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TOPIC

Characterization of cereal *Fusarium* species resistant to major seed disinfection fungicides

Presented by: BELABED Imane

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University Year 2023/2024

In memory of my dear late Father

“May GOD grant Him with His Grace and Holy Mercy“

To my dear Mother

To my beloved sisters

To all my wonderful colleagues and friends

List of publications

Belabed, I., Abed, H., Bencheikh, A., & Rouag, N. (2022). *Fusarium* species associated with wheat head blight disease in Algeria: characterization and effects of triazole fungicides. *Pesticides and Phytomedicine/Pesticidi i fitomedicina*, *37*(2), 49-62. DOI: https://doi.org/10.2298/PIF2202049I

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Bencheikh, A., Rouag, N., Mamache, W., & **Belabed, I.** (2020). First report of *Fusarium equiseti* causing crown rot and damping-off on durum wheat in Algeria. *Archives of Phytopathology and Plant Protection*, *53*(19-20), 915-931. DOI: https://doi.org/10.1080/03235408.2020.1804303

Belabed, I., Abed, H., Bencheikh, A., Carolina, M.S., Cunha, S., Pinto, E., & Rouag, N. (2024). Pathogenicity and mycotoxin profile of *Fusarium* spp. inducing wheat head blight in Algeria. *Mycotoxin Research.* In progress.

Belabed, I., Sadrati, N., Bencheikh A., Abed, H., & Rouag, N. (2024). Diversity of *Fusarium* species complex, the causal agents of FHB disease of wheat in Algeria. *Canadian Journal of Plant Pathology.* In progress.

Belabed, I., Abed, H., Bencheikh, A., & Rouag, N. (2023). Multiple trophic and climatic factors' impacts on kinetics *Fusarium* spp. growth involved in Fusarium wilt in wheat. *Journal of Crop Protection.* In progress.

Belabed, I., Bencheikh, A., & Rouag, N. (2024). Fusarium head blight of wheat: Current knowledge on associated species and their mycotoxins, pathogenicity diversity, and management strategies. *Australasian Plant Pathology.* In progress.

List of communications

Belabed, I., Abed, H., Bencheikh, A., & Rouag, N. Study of the pathogenicity of *Fusarium* spp. isolates on the basal part of some varieties of durum wheat (*Tritucum durum*) in Algeria. 1st International conference on applied engineering and natural sciences (Webinar). 1-3, November, 2021, Konya. Turkey.

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Belabed, I., Abed, H., Bencheikh, A., & Rouag, N. Toxigenic moulds in Algerian wheat intended for human consumption. 1st International days of natural and life sciences (Webinar). 1-2, March, 2022, Ouargla. Algeria.

Belabed, I., Abed, H., Bencheikh, A., & Rouag, N. Study of the pathogenicity of several *Fusarium* species on the growth of coleoptiles and roots *in vitro* of some durum wheat varieties (*Triticum durum*).1st International webinar of animal biodiversity, protection and environment. 26-27, May, 2022, Algiers. Algeria.

Belabed, I., Abed, H., Bencheikh, A., Bourdim, H., Sebaat, I., Zerguine, I., & Rouag, N. Study of *Fusarium* isolates effect on the vegetative and root development of some durum wheat varieties (*Triticum durum*). 1st National seminar on the valorisation of natural resources and the environment (Webinar). 30, March, 2022, Sétif. Algeria.

Belabed, I., Abed, H., Bencheikh, A., & Rouag, N. Mycotoxins and mycotoxigenic fungi (*Fusarium* spp.) in Algerian wheat, a risk to human health. National seminar on food and health (Webinar). 05, December, 2022, Relizane. Algeria.

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Abstract

Fusarium species are known to be major producers of mycotoxins, causing Fusarium head blight (FHB) disease, which reduces wheat yield and quality. To complete this study, 60 samples of durum wheat with symptoms of the disease, belonging to six varieties, were collected randomly from seven provinces in northeastern Algeria during the years 2017 and 2018 to determine the pathogens prevalence and their importance, the association of the pathogens with nutritional and climatic factors, as well as the effectiveness of fungicides to their control. Eighteen fungal isolates were isolated and then identified as belonging to seven different species of *Fusarium*, namely *F. clavum*, *F. culmorum*, *F. microconidium*, *F. avenaceum*, *F. tricinctum, F. solani*, and *F. acuminatum* using polyphasic analysis. The results showed that *F. clavum* was the most abundant, found in 33.3% of the samples. This study also indicates the presence of *F. clavum*, *F. microconidium*, and *F. tricinctum*, for the first time in durum wheat ears in Algeria, and *F. microconidium* in durum wheat worldwide. The pathogenicity of isolates on three durum wheat cultivars (GTAdur, Cirta, and Waha) was evaluated using *in vivo* and *in vitro* tests, which showed a significant difference between isolates and between species, with *F. avenaceum* (FusBi7) being the most aggressive, and Cirta variety as the most tolerant. Regarding mycotoxins production, all isolates tested were able to produce deoxynivalenol (DON), zearalenone (ZEA), and T-2 toxin (T-2), with the distinction of *F. culmorum* (FusBo59) as being the most productive. In addition, the results showed that the DON is the most abundant with 7.128 $\mu g.kg^{-1}$ recorded by ELISA and 373196.19 $\mu g.kg^{-1}$ by LC-MS/MS, exceeding the European Commission limits (1750 $\mu g.kg^{1}$). On the other hand, the analysis of the toxins presents in the durum wheat samples revealed that 15-ADON was more present (63.6%) than DON (18.2%) and 3-ADON (9.1%). The study also showed that physical and chemical factors, as well as nutritional factors, greatly affect the growth ability of the isolates. Laboratory studies using a one-factor method at a time to understand the physiological aspect of *Fusarium* isolates indicated that Czapek Dox Agar, at 25°C, 95% relative humidity, pH 7, 2.5 g. L^{-1} of salinity, cellulose as a carbon source, peptone as a nitrogen source and a 10:1 ratio of C:N, recorded optimal fungal growth for the *Fusarium* isolates. Antifungal activity assays also demonstrated that the fungicide tebuconazole (Raxil and Tebuzol) and the combination of fludioxonil + difenoconazole significantly inhibited the fungal growth of the isolates by 84.31%, 82.94%, and 81.33%, respectively, compared to difenoconazole alone (73.16%) at the recommended dose after five days of exposure. Tebuconazole (Tebuzol 73.46%, Raxil 69.75%) had a greater effect on spore germination than fludioxonil + difenoconazole (62.16%) at the recommended dose leading to conidial deformation and fragmentation.

Keywords: Epidemiology, wheat, *Fusarium* spp., mycotoxins, fungicides.

الملخص

تُعرف أنواع الفيوزاريوم بأنها منتج رئيسي للسموم الفطرية، مما يسبب مرض فيوزاريوز سنابل القمح، الذي يقلل من إنتاجية القمح وجودته. لإنجاز هذه الدراسة تم جمع 60 عينة من القمح الصلب المصحوب بأعراض المرض، تنتمي الى ستة أصناف، بشكل عشوائي من سبع ولايات من الشمال الشرقي الجزائري خلال عامي 2017 و2018 لتحديد مدى انتشار مسببات المرض وأهميتها، ارتباط مسببات المرض بالعوامل الغذائية والمناخية، وكذلك مدى فعالية مبيدات الفطريات لمكافحتها. تم عزل 18 عزلة فطرية، ومن ثم تم تعريفها على أنها تنتمي الى سبعة أنواع مختلفة من جنس الفيوزاريوم، وهي *F. clavum*، *F. culmorum*، *F. microconidium*، *F. avenaceum*، *F. tricinctum*، *F. solani*، و*F. acuminatum* باستخدام التحليل متعدد الأطوار. أظهرت النتائج أن *F. clavum* هو الأكثر تواجدا بنسبة 33.3% كما كشفت هذه الدراسة عن وجود *F. clavum*، *F. microconidium*، و*F. tricinctum*، لأول مرة في سنابل القمح الصلب في الجزائر، و*F. microconidium* في القمح الصلب في جميع أنحاء العالم. تم تقييم القدرة الإمراضية للعزلات على ثلاثة أصناف من القمح الصلب (GTAdur، Cirta، وWaha) باستخدام الاختبارات *in vivo* و*in vitro* والتي أظهرت اختلافًا كبيرًا في القدرة الإمراضية بين العزلات وبين الأنواع، حيث كانت *F. avenaceum* (FusBi7) هي الأكثر عدائية، وكان صنف Cirta هو الأكثر تحملاً. جميع العزلات التي تم اختبارها للتأكد من قدرتها على إنتاج السموم الفطرية انتجت ديوكسينيفالينول (DON)، وزيرالينون (ZEA)، وتوكسين T-2 (T-2) ، مع كون *F. culmorum* (FusBo59) هي الأكثر سمية، أدت إلى إنتاج DON باعتباره هو الأكثر وفرة مع تسجيل أقصى تركيز له 7.128 ميكروجرام/كغ في اختبار ELISA و373196.19 ميكروجرام/ كغ بواسطة LC-MS/MS، وهو ما يتجاوز حدود المفوضية الأوروبية (1750 ميكروجرام/ كغ). كما أن تحليل السموم المتواجدة داخل عينات القمح الصلب أظهر النتائج أن ADON-15 كان الأكثر وجودا (63.6%) من DON (18.2%) و3-ADON (9.1%). كما بينت الدراسة أن العوامل الفزيائية والكيميائية وكذا الغذائية تؤثر بشكل كبير على قدرة نمو العزلات وأشارت الدراسات المختبرية باستخدام طريقة العامل الواحد في كل مرة لفهم الجانب الفسيولوجي لعزلات الفيوزاريوم ان Czapek Dox Agar، عند25 °C، 95٪ من الرطوبة النسبية، درجة الحموضة 7، 2.5 غ / ل من الملوحة، السليلوز كمصدر للكربون، الببتون كمصدر للنيتروجين ونسبة 10: 1 ل C: N، سجلوا نمو فطري أمثل لعزلات الفيوزاريوم. كما اظهرت اختبارات النشاط المضاد للفطريات أن المبيد الفطري تيبوكونازول (راكسيل وتيبوزول) ومزيج فلوديوكسونيل + ديفينوكونازول ثبطوا بشكل كبير من النمو الفطري للعزلات بنسبة 84.31%، 82.94%، 81.33%، على التوالي، مقارنة بالديفينوكونازول لوحده (73.16%) عند الجرعة الموصى بها بعد خمسة أيام من التعرض. كان للتبوكونازول (تيبوزول 73.46%، راكسيل 69.75%) تأثير أكبر على إنبات الابواغ من فلوديوكسونيل + ديفينوكونازول (62.16%) عند الجرعة الموصى بها، مما ادي إلى تشوه وتفتت الابواغ.

الكلمات المفتاحية: علم الأوبئة، القمح، أنواع الفيوزاريوم، السموم الفطرية، مبيدات الفطريات.

Résumé

Les espèces de *Fusarium* sont connues pour être d'importantes productrices de mycotoxines, provoquant la brûlure de l'épi (FHB), qui réduit le rendement et la qualité du blé. Pour compléter cette étude, 60 échantillons de blé dur présentant des symptômes de la maladie, appartenant à six variétés, ont été collectés de manière aléatoire dans sept Wilayas du nord-est de l'Algérie au cours des années 2017 et 2018 afin de déterminer la prévalence des pathogènes et leur importance, l'association des pathogènes avec les facteurs nutritionnels et climatiques, ainsi que l'efficacité des fongicides pour leur contrôle. Dix-huit isolats fongiques ont été isolés puis identifiés comme appartenant à sept espèces différentes de *Fusarium*, à savoir *F. clavum, F. culmorum, F. microconidium, F. avenaceum, F. tricinctum, F. solani* et *F. acuminatum* par analyse polyphasique. Les résultats ont montré que *F. clavum* était le plus abondant, présent dans 33.3% des échantillons. Cette étude indique également la présence de *F. clavum*, *F. microconidium* et *F. tricinctum*, pour la première fois dans les épis de blé dur en Algérie, et de *F. microconidium* dans le blé dur dans le monde. La pathogénicité des isolats sur trois variétés de blé dur (GTAdur, Cirta et Waha) a été évaluée à l'aide de tests *in vivo* et *in vitro*, qui ont montré une différence significative entre les isolats et entre les espèces, *F. avenaceum* (FusBi7) étant le plus agressif, et la variété Cirta comme la plus tolérante. Concernant la production des mycotoxines, tous les isolats testés étaient capables de produire du déoxynivalénol (DON), de la zéaralénone (ZEA) et de la toxine T-2 (T-2), avec la distinction de *F. culmorum* (FusBo59) comme étant le plus productif. De plus, les résultats montrent que le DON est le plus abondant avec 7.128 $\mu g.kg^{-1}$ enregistrés par ELISA et 373196.19 $\mu g.kg^{-1}$ par LC-MS/MS, dépassant les limites de la Commission Européenne (1750 $\mu g.kg^{-1}$). D'autre part, l'analyse des toxines présentes dans les échantillons de blé dur a montré que le 15-ADON était le plus présent (63.6%) que le DON (18.2%) et le 3-ADON (9.1%). L'étude a également montré que les facteurs physiques et chimiques, ainsi que les facteurs nutritionnels, affectent significativement la capacité de croissance des isolats. Des études en laboratoire ont indiqué qu'à travers l'utilisation d'une méthode à un facteur à la fois pour comprendre l'aspect physiologique des isolats de *Fusarium,* le Czapek Dox Agar, à 25°C, 95% d'humidité relative, pH 7, 2.5 g. L^{-1} de salinité, cellulose comme source de carbone, peptone comme source d'azote et un rapport C:N de 10 : 1, enregistraient une croissance fongique optimale pour les isolats de *Fusarium*. Les tests d'activité antifongique ont également montré que le fongicide tébuconazole (Raxil et Tebuzol) et l'association fludioxonil + difénoconazole inhibaient de manière significative la croissance fongique des isolats de 84.31%, 82.94% et 81.33%, respectivement, par rapport au difénoconazole seul (73.16%) à la dose recommandée après cinq jours d'exposition. Le tébuconazole (Tebuzol 73.46%, Raxil 69.75%) a eu un effet plus important sur la germination des spores que le fludioxonil + difénoconazole (62.16%) à la dose recommandée, entraînant une déformation et une fragmentation des conidies.

Mots clés : Épidémiologie, blé, *Fusarium* spp., mycotoxines, fongicides.

ABBREVIATIONS

15-ADON	15-acetyldeoxynivalenol
3-ADON	3-acetyldeoxynivalenol
4ANIV	4 Acetylnivalenol
acl1	ATP citrate lyase
ACU	Acuminatopyrone
AE	Anther extrusion
AFB1	Aflatoxin-B1
AFB2	Aflatoxin-B2
AFG1	Aflatoxin-G1
AFG2	Aflatoxin-G2
Afs	Aflatoxins
AHBWP	Area harvested for bread wheat in each province
AHDWP	Area harvested for durum wheat in each province
ANOVA	Analysis of variance
AT	Aminotransferase
AUDPC1	Area under disease progress curve
BCAs	Biological control agents
BEA	Beauvericin
BLAST	Basic local alignment search tool
BLAST-N	Basic local alignment search tool nucleotide
BS	Bootstrap
BUT	Butanolide
BW	Bread wheat
BWPP	Bread wheat production by province
CaM	Calmodulin
cAMP-PKA	Cyclic AMP-protein kinase A
CDA	Czapek dox agar
CHL	Chlamydosporol
CL	Coleoptile length
CLA	Carnation leaf agar
CMA	Corn meal gar
CNCC	National center for certification and control of seeds and plants
CUL	Culmorin
CYA	Cezapec yeast agar
DAS	Diacetoxyscirpenol
Dc	Diameter of colony in control
DMI	Demethylation inhibitors
DON	Deoxynivalenol
DPI	Days post inoculation
DPPTC	Direction of plant protection and technical controls
Dt	Diameter of colony in treatment
DW	Durum wheat
DWPP	Durum wheat production by province
EC	European commission
EHP	Eastern high plains
ELISA	Enzyme-linked immunosorbent assay
ENNs	Enniantins
EU	European union

FAO	Food and agriculture organization of the United Nations
FB1	Fumonisin-B1
FB2	Fumonisin-B2
FBs	Fumonisins B
FCR	Fusarium crown rot
FCs	Fumonisins C
FHB	Fusarium head blight
FOL	*F. oxysporum* f. sp. *Lycopersici*
FRR	Fusarium root rot
FTF	Fusarium transcription factor
FUMS	Fumonisins
FUNG	Fungerin
FUS	Fusaproliferin
FWVS	Fresh weight of vegetative system
GR	Germination rate
HPLC	High-performance liquid chromatography
HT-2	HT-2 toxins
IMP	Integrated management program
IPM	Integrated pest management
ITS	Internal transcribed spacer
LC-MS/MS	Liquid chromatography coupled to tandem mass spectrometry
LOD	Limits of detection
LONG	Longiborneol
LOQ	Limit of quantitation
LSU	28S large subunit of the nrDNA
MAPK	Mitogen-activated protein kinase
MARD	Ministry of agriculture and rural development
MGI	Mycelial growth inhibition
ML	Maximum likelihood
MLST	Multilocus sequence typing
MON	Moniliformin
MRM	Multiple reactions monitoring
MS	Mass spectrometric
NCBI	National center for biotechnology information
NIV	Nivalenol
OHCUL	Hydroxy culmorin
ORFs	Open reading frames
OTA	Ochratoxin A
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PH	Plant height
PHI-base	Pathogen–host interactions Database
PKS	Polyketide synthase
PP	Phenylpyrroles
PSA	Potato sucrose agar
QoI	Quinone outside inhibitors
qPCR	Quantitative polymerase chain reaction
QTLs	Quantitative trait loci
QuEChERS	Quick, easy, cheap, effective, rugged and safe

RH	Relative humidity
RPB1	RNA polymerase I large subunit 1
RPB2	RNA polymerase II large subunit 1
RSD	Relative standard deviation
RSL	Root system length
RSW	Root system weight
SAB	Sabouraud dextrose agar
SBI	Sterol biosynthesis inhibitors
SDHIs	Succinate dehydrogenase inhibitors
SDR	Short-chain dehydrogenase reductase
SIX	Secreted in xylem
SNA	Spezieller nährstoffarmer agar
SP chromosomes	Supernumerary chromosomes
SRN	Seminal root number
STB	Septoria tritici blotch
T-2	T-2 toxins
TCA	Tricarballylic acid
TCT	Trichothecenes
TCTA	Trichothecenes type A
TCTB	Trichothecenes type B
TEF1-α or TEF1	Translation elongation factor 1-alpha
TLC	Thin layer chromatography
TRI	Trichothecenes
tub2	Beta-tubulin
UAA	Useful agricultural area
UV	Ultraviolet
VSL	Vegetative system length
VSW	Vegetative system weight
WB	Wheat blast
WGEA	Wheat grain extract agar
Z14G	Zearalenone-14-Glucoside
Z14S	Zearalenone-14-sulphate
Z16G	Zearalenone-16-β-D-glucopyranoside
ZEA	Zearalenone
α -ZAL	α-Zearalanol
α-ZEL	α-Zearalenol
β -ZAL	β-Zearalanol
β-ZEL	β-Zearalenol

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General introduction

Wheat is one of the most important staple food crops worldwide and one of the cornerstones of global food security. The crop ranks second place in cereal production after coarse grain, with a production of 785 million tons (MT) on a harvested area of 219 million hectares (Mha) in 2023 (FAO, 2023).

In Algeria, wheat occupies a strategic place in the country's diet and economy, currently cultivated on about 2 Mha nationwide, with production forecast of 2.7 MT in 2023/24. In light of rising demand for wheat, Algeria is suffering from a production shortfall. Each year, this shortfall is supplemented by imports to meet national consumption demands, wheat imports in 2023–2024 are expected to reach 8.7 MT (MARD, 2023).

Wheat production is affected by biotic (pathogens and pests) and abiotic (drought and heat) stresses. Among biotic stresses, diseases caused by pathogens, which include fungi, bacteria and viruses, may be responsible for an average global loss of 21.5% of wheat production (Savary *et al*., 2019).

Fusarium head blight (FHB) is an economically important fungal disease of various food and feed crops, like wheat and is well known in wheat-growing areas in Algeria and worldwide (Abdallah-Nekache *et al*., 2019). It is caused by a complex of diverse *Fusarium* species that are spread over different geographical regions and have responded to various climates (Xu *et al*., 2008). These fungi are also facultative saprophytes that survive and overwinter on crop residues and debris from previous years (Schmale and Bergstrom, 2010), which serve as the initial source of inoculum for new epidemics. Other sources of inoculum involve the soil, infected seeds, and numerous host plants.

When environmental factors are conducive, such as moderate to high temperatures, high humidity, and light during wheat anthesis, *Fusarium* infection occurs, the disease spreads within the ears, and mycotoxins accumulate. *Fusarium* spp. contamination is an extremely significant problem for global agriculture, reducing grain quality and yield, as evidenced by size, weight loss, discoloration, shrivelling, carbohydrate and protein composition changes, and mycotoxins occurrence (Magliano and Kikot, 2013). Mycotoxins accumulating in infected grains act as virulence factors that exhibited undeniable toxicological impacts on human and animal health (Gong *et al*., 2015). The major fusarial toxins are trichothecenes, zearalenones, fumonisins, and the emerging toxins include enniatins, beauvericin, fusaproliferin, and moniliformin (Ferrigo *et al*., 2016).

FHB pathogen species can be classified based on the profile of toxic secondary metabolites produced, which can result in diverse chemotype profiles that can cause different forms of grain infection depending on the occurrence of each species in the crop. Recently, several reports indicated that *Fusarium culmorum* was the most common and harmful species of the FHB complex recovered in Algerian wheat (Abdallah-Nekache *et al*., 2019; Hadjout *et al*., 2022) and has produced multiple mycotoxins like deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), zearalenone (ZEA), and nivalenol (NIV) (Belabed *et al*., 2023; Hadjout and Zouidi, 2023). Due to detrimental impact on consumer health, several countries have set thresholds for toxin levels in feed and food items, and even more worryingly**,** no strict regulation has yet been established in Algeria.

FHB is also known as a "complicated" disease for many reasons, including diversity of *Fusarium* species associated with this disease in addition to two *Microdochium* species, mycotoxin content, huge yield losses, reduction in seed quality, lack of a fully effective control methods, lack of FHB-resistant wheat varieties, pathogen's ability to attack different parts of the wheat plant (head, crown and root), pathogen population dynamics, and climate change. Given the extreme toxicity of *Fusarium* mycotoxins and the impact of FHB on wheat yields, it is critical that control measures for this disease be figured out. Recommended approaches to controlling FHB disease include the use of FHB-resistant wheat varieties, biological control, fungicides, appropriate cultural practices, and crop rotation (Mesterházy *et al*., 2015), rather, an integrated management strategy is a better option to protect wheat crops from the pathogen. The most widely used control method involves using a correct application of fungicide combined with moderately resistant varieties; nonetheless, severe disease severity is frequently observed when there is intense pathogen invasion and suitable environmental conditions. Further research is therefore required to develop new and enhanced approaches.

Despite the increasing occurrence of these pathogens in wheat fields and their potential to cause severe losses in both yield and quality, knowledge of their distribution, importance, ecophysiology profile, and mitigation measures is lacking in Algeria. FHB of wheat surveys carried out in several wheat-growing regions of Algeria have shown that the disease appears to be an important constraint on wheat production (Hadjout *et al*., 2022). Therefore, promptly resolving the problem of this emerging disease would support attempts to overcome Algeria's food security challenges. That is why this prospective study

was designed to obtain data which will help to address these research gaps by meeting the following objectives:

- Assessment of the phylogenetic diversity and phenotypic variability of different isolates related to FHB of durum wheat in Algeria.
- Evaluation of *Fusarium* isolate pathogenicity profiles using *in vitro* and *in vivo* tests.
- Evaluation of mycotoxin levels in culture of toxigenic *Fusarium* spp. as well as in several durum wheat grain samples using ELISA kit and LC-MS/MS methods.
- Studying the correlation between pathogenicity and mycotoxins production.
- Assessment of pathogen growth under different ecophysiological conditions corresponding to potential climate change scenarios, with the aim of gathering empirical knowledge to improve prevention and control strategies of mycotoxin and yield loss risks in Algerian wheat crops.
- Studying the efficacy of triazole fungicides *in vitro* on mycelial growth and spore germination of FHB isolates.

1. Literature review

1.1. Host plant: durum wheat (*Triticum durum*)

1.1.1. Economic importance of the durum wheat crop

1.1.1.1. On a worldwide scale

Wheat is the second most cultivated cereal in the world after corn (FAO, 2023). Durum wheat (DW) (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) plays a major role for agro-economy development with over 16 million hectares planted and 38 million tonnes of grain produced annually (Martínez-Moreno *et al*., 2020), representing for less than 7% of the worldwide wheat production (Martínez-Moreno, 2022).

Table 1.1. World total grains (wheat and coarse grains) production in Million Tons during the period 2019 to 2023.

Crops	2019/2020	2020/2021	2021/2022	2022/2023
Wheat	761	773	781	796
Maize (corn)	1132	1136	1219	1161
Soybean	341	370	356	385
Rice	500	510	516	504
Total grains	**2193**	**2227**	**2290**	**2256**

Source: International Grains Council (IGC, 2023).

Indeed, the countries of the Mediterranean basin (Algeria, Turkey, Italy, Morocco, Syria, Tunisia, France, Spain, and Greece) cover about 50% of the world's area and production (Martínez-Moreno, 2022). Moreover, Canada, Mexico, the USA, Russia, Kazakhstan, Azerbaijan, and India are substantial to considerable DW producers, with the first three are the most prominent DW exporters (Martínez-Moreno, 2022) (Figure 1.1).

1.1.1.2. In Algeria

Cereal agriculture, dominated by durum wheat, holds a pivotal place in the food system and the national economy of Algeria, by the importance of the acreages which accounts for 85% of the useful agricultural area (UAA) and is situated in the high plains region, distinguished by semi-arid climate (Kourat, 2021). Due to climate change, the water cycle has been modified, leading to degradation of agricultural land, a decrease in agricultural production and yields, and a failure in biodiversity (Bessaoud *et al*., 2019). In light of the drop in oil prices, Algeria's economic balance and food security may now be seriously threatened by the country's failure to fulfill its national wheat demand (Harrag and Boulfred, 2019).

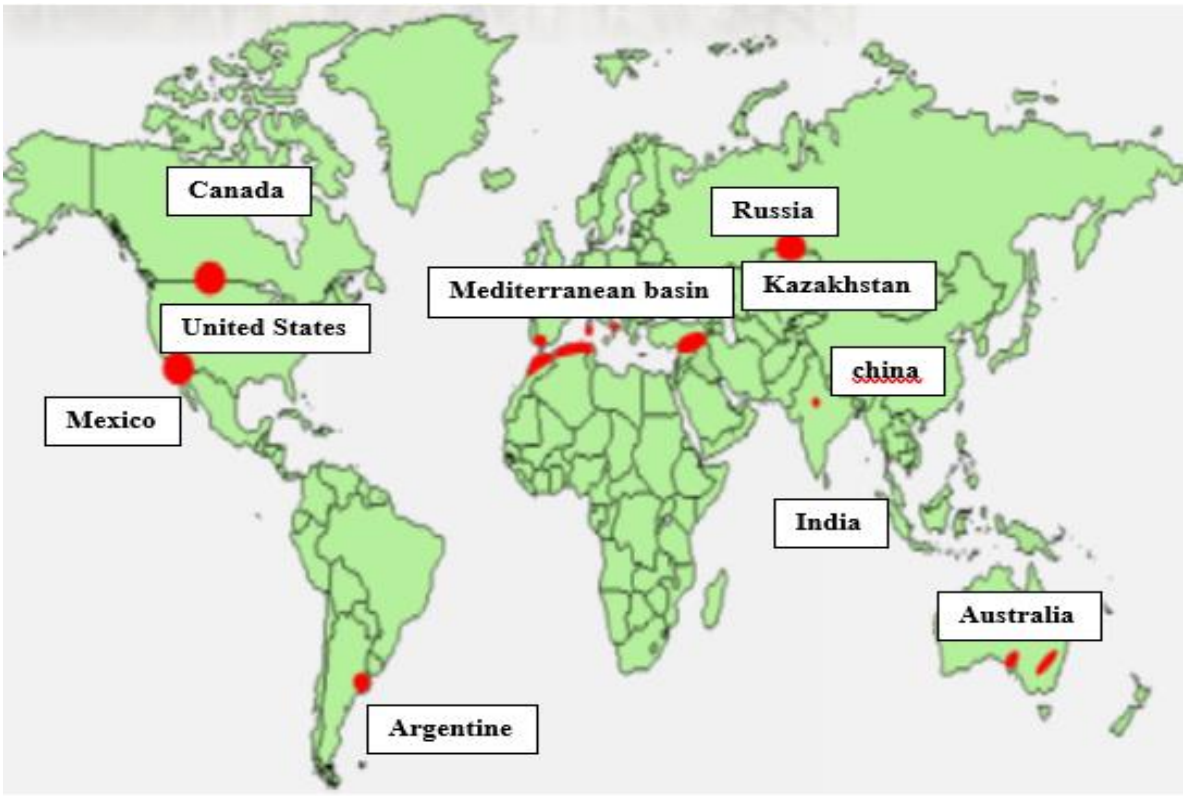


Figure 1.1. Map showing the main durum wheat cultivating countries (Ranieri, 2015).

According to statistical data provided by MARD (2020), the eastern high plains (EHP) provinces including Batna, Constantine, Khenchla, Setif, Oum El Bouaghi, Tebessa …as well as the central plains province of Medea, the western plains province of Chlef, and the western high plains provinces including Tiaret and Relizane, place a high value on the areas harvested in wheat (bread and durum). Except for the provinces of Mascara, Saida and Sidi Bel Abes, where the $AHBW_P$ is greater than the $AHDW_P$, the $AHBW_P$ keeps going to be lesser than the $AHDW_P$ (Figure 1.2a).

Following that of the harvested regions is the distribution of the average production of DW (DWP_P) and BW (BWP_P). The East of the country's provinces, particularly those in the EHP like, Guelma, Souk Ahras and Setif, are where one may find the best DWP_P (Figure 1.2b).

1.1.2. Biotic and abiotic constraints of the durum wheat crop

The requirement for durum wheat grains will increase daily, however due to multiple biotic and abiotic factors, both its quality and yield may drastically deteriorate causing a threat for the world's food security. Abiotic stresses primarily drought and heat (Dettori *et al*., 2022), salinity stress (Soni *et al*., 2022), heavy metals (Shah T *et al*., 2018), chilling and

other factors can arise through various wheat growth phases leading to substantial yield losses. Biotic stresses can also greatly reduce durum wheat productivity through several

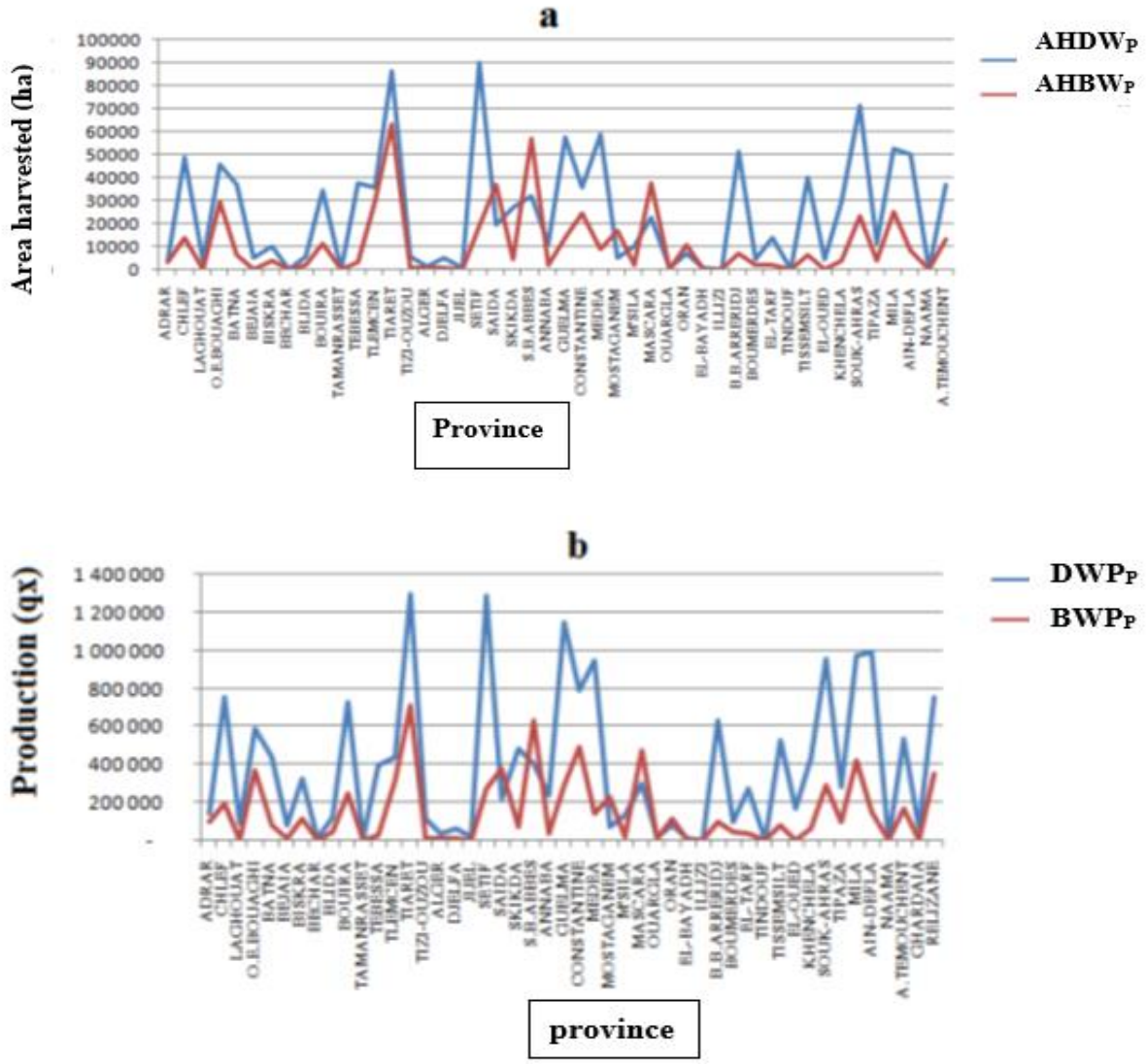


Figure 1.2. Importance of wheat cultivation in Algeria during the period 1998-2019. (a) Variability of harvested areas, and (b) Variability of durum and bread wheat production (Kourat, 2021).

fungal pathogen species inducing severe diseases worldwide like leaf rust (*Puccinia recondita*; synonym *Puccinia triticina*), stripe rust or yellow rust (*Puccinia striiformis* Westend. f. sp. *tritici* Erikss), Powdery mildew (*Blumeria graminis* f.sp. *tritici*), *Septoria nodorum* blotch (*Parastagonospora nodorum*; synonym *Septoria nodorum*), and *Septoria tritici* blotch (STB) (*Zymoseptoria tritici*; synonym *Septoria tritici*) are the most prevalent foliar wheat diseases in Europe (Willocquet *et al*., 2021), wheat blast (WB) (*Magnaporthe*

oryzae pathotype *Triticum* (anamorph *Pyricularia oryzae* pathotype *Triticum*)) (Islam *et al*., 2019; Singh *et al*., 2021), Stem rust (*Puccinia graminis* f. sp. *tritici*) (Marone *et al*., 2022), Fusarium head blight (*Fusarium* spp.) (Goswani and Kistler, 2004).

Acknowledging how biotic and abiotic factors affect the Algerian durum wheat production can help us better comprehend their agro-economic outcomes for the country's food security. So, with respect to abiotic factors, are climatic (increased temperatures, decreased precipitation, and net solar radiation) (Kourat *et al*., 2022) or edaphic (soil acidity). Unfortunately, recent climatic changes have worsened these detrimental effects on durum wheat yield. Additionally, the biotic factors are exhibited in several aggressions of various nature which are typically weeds (wild oats, brome, Phalaris, poppy, medicago), pests (insects, birds and rodents), pathogens (fungi, bacteria, viruses and nematodes). Durum wheat in Algeria is susceptible to several diseases, which can greatly decrease grain output, including fungal diseases could be categorized based on the symptoms they produce and the parts they damage (Aouali and Douici-Khalfi, 2009). Therefore, we distinguish diseases causing:

- **Localized symptoms on the leaves:** leaf rust (*Puccinia recondita*; synonym *Puccinia triticina*), stripe rust or yellow rust (*Puccinia striiformis),* Oidium *(Erysiphe graminis* f.sp. *tritici)* (Aouali and Douici-Khalfi, 2009), Septoria leaf blotch (*Zymoseptoria tritici*) (Harrat *et al*., 2017), septorian spot (*Mycosphaerella graminicol*) (Ayad *et al*., 2011), helminthosporium spot (*Pyrenophora tritici-repentis* (Died) Drechs) (Benslimane *et al*., 2011).
- **Root rot:** Fusarium crown rot (FCR; also known as foot and root rot) (*F. culmorum* and *F. pseudograminearum*) (Yekkour *et al*., 2015); Foot rot – scalding (*Gaeumannomyces graminis)* (Aouali and Douici-Khalfi, 2009).
- **Symptoms on the heads:** Fusarium head blight (FHB) (*F. culmorum*) (Touati-Hattab *et al*., 2016), caries (*Tilletia foetida*) (Aouali and Douici-Khalfi, 2009).

Noteworthy, FCR and FHB are the most harmful crop diseases in the country (Yekkour *et al*., 2015).

1.2. Fusarium head blight of wheat

Fusarium head blight (FHB) is a fungal disease targeting predominantly host plant species include wheat (*Triticum* spp.), barley (*Hordeum* spp.), rice (*Oryza* spp.), maize (*Zea* spp.), rye (*Secale cereale*), triticale *(×Triticosecale* spp.) and oats (*Avena* spp.) (Chen *et al*.,

2022). It is due to several *Fusarium* species and is known as the “cancer” of wheat (Hu *et al*., 2022).

1.2.1. Economic importance

FHB is typically rated as the fourth most significant plant fungal disease worldwide in terms of both science and economic (Dean *et al*., 2012). FHB has an adverse economic impact, because of lost production, mycotoxin contamination, human health costs, and decreased livestock productivity (Bacon and Hinton, 2007; Matny, 2015). In China, FHB yearly harmed upwards of 4.5 million hectares of wheat fields, amounting for nearly 20% of the total wheat acres during 2000, and yield losses due to this disease amounted to more than 3.41 million tons annually (Chen *et al*., 2019). Losses in United States by FHB in wheat and barley during 1990’s were over 3 billion $ (Windels, 2000). Up to 70% of Argentina's output losses were attributed to FHB in 2012, while from 2000 to 2010, yield losses in southern Brazil fluctuated from 11.6% to 39.8% (Reis and Carmona, 2013).

1.2.2. Aetiological complexity

The disease complex is associated with at least nineteen *Fusarium* species, and two causative agents of *Microdochium*, while *F. culmorum*, *F. graminearum*, and *F. avenaceum*, are the most hazardous and prevalent species globally (Teli *et al*., 2020). Nevertheless, when environmental conditions are unfavorable for the growth of the primary FHB casual agents, further species, such as *Fusarium sporotrichioides* Sherb., *Fusarium crookwellense*, *Fusarium roseum* Link (syn. *F. cerealis* (Cooke) Sacc.), *Fusarium equiseti* (Corda) Sacc., *Fusarium tricinctum* (Corda) Sacc., *Fusarium oxysporum* Schltdl., and *Fusarium langsethiae*, might contribute significantly to disease development (Mielniczuk and Skwaryło-Bednarz, 2020). There are three new species that can induce FHB, including *F. dactylidis* (Aoki *et al*., 2015), *F. praegraminearum* (Gräfenhan *et al*., 2016), and *F. subtropicale* (Pereira *et al*., 2018) have recently been reported.

1.2.3. Geographical distribution of FHB pathogens

Occurrence and severity of FHB as well as *Fusarium* species populations fluctuate throughout geographic locations (Doohan *et al*., 1998), host plant (Van der Lee *et al*., 2015) and years attributed to differences in agriculture techniques and climatic patterns (Klix *et al*., 2008). The most important climate change impacts are shifts in the geographical distribution of pathogens (Panwar *et al*., 2016), as well as the creation of optimal conditions for further population shifts like replacement of *F. graminearum* by *F.*

poae in the Czech Republic (Sumíková *et al*., 2017), in Italy (Valverde-Bogantes *et al*., 2020), replacement of *F. culmorum* by *F. graminearum* in USA (Bissonnette *et al*., 2018), in Europe (Valverde-Bogantes *et al*., 2020).

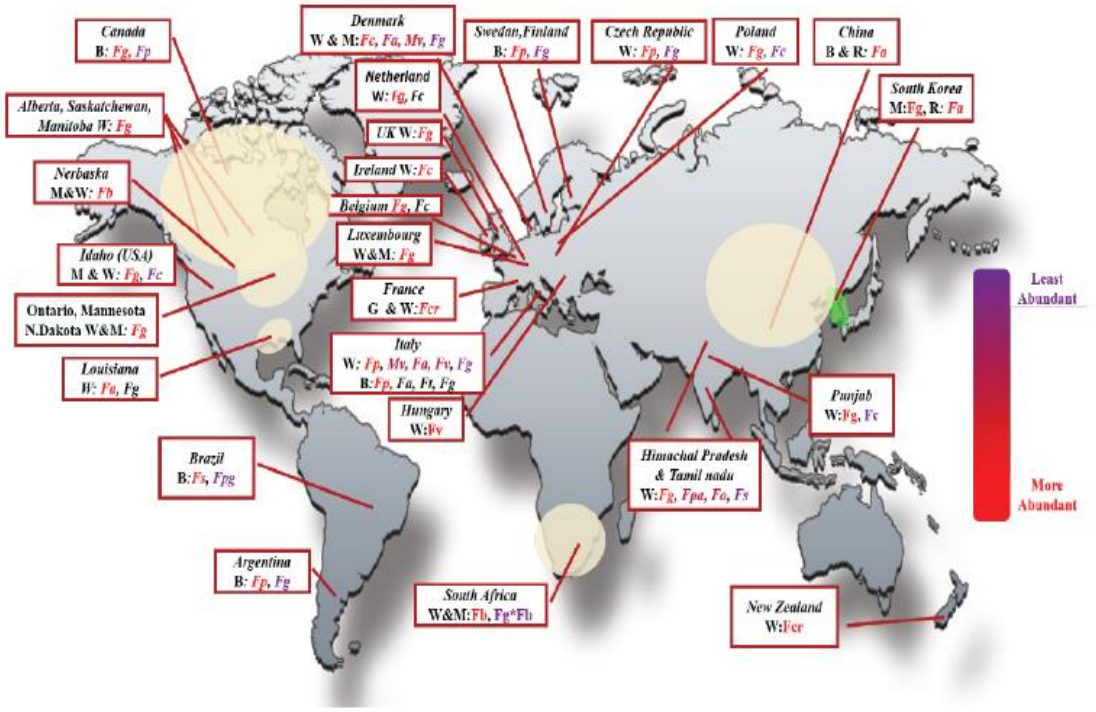


Figure 1.3. Map showing the global distribution of Fusarium head blight associated pathogens. The predominance of *F. graminearum* from tropical to temperate climate is indicated. Fpa: *F. pallidoroseum*, Fg: *F. graminearum*, Fc: *F. culmorum*, Fo: *F. oxysporum*, Fe: *F. equiseti*, Fa: *F. asiaticum*, Fsa: *F. semitectum*, Fcr: *F. cortaderiae*, Fb: *F. boothii*, Fv: *F. vorosii*, Fp: *F. poae*, Mv: *M. nivale*; W: Wheat, B: Barley, M: Maize, R: Rice (Teli *et al*., 2020).

1.2.4. Disease epidemiology

FHB is a monocyclic disease. The overall disease cycle and symptoms of FHB on wheat spikes and kernels, are depicted in Figure 1.4. The pathogen survives mainly in crop wastes or soil as ascospores, which are sexual structures called perithecia, or as asexual spores called macroconidia or microconidia for species with only an anamorphic stage (Alisaac and Mahlein, 2023).

The spores are the initial inoculum of the disease. Conditions which promote inoculum production are warm temperatures and high relative humidity (Saharan *et al*., 2021). Ascospores are generated at temperatures between 13 and 33°C, while macroconidia can produce in the range of 16 to 36°C, with 32°C considered optimal (Leslie and Summerell,

2008; Saharan *et al*., 2021). The pathogen afterwards begins to produce globose, asexual, thick-walled spores known as chlamydospore, and also produces perithecia, which are dark purple or black sexual fruiting structures in its teleomorphic stage, from which forcefully releases mature ascospores into the air (Teli *et al*., 2020).

At wheat anthesis, which is the infection-prone stage, and under suitable climatic conditions, airborne spores are disseminated to healthy plants by wind or rain-splash after overwintering and land on spikelets. On the spikelet tissue, the spores germinate and form germination tubes. Following germination, the fungal hyphae expand on the ovary, palea, and lemma's surface and begin to secrete mycotoxins without penetrating the spikelet tissue. the pathogen then penetrates the host tissue and initiates a biotrophic infection with an intercellular growth in the spikelet before progressing to a necrotrophic stage with inter- and intracellular growth laterally and vertically within the spike (Alisaac and Mahlein, 2023) (Figure 1.4).

Due to the accumulation of mycotoxins at this stage of pathogenicity in both the spike tissue and the kernels, crop yield and quality are decreased (Kang and Buchenauer, 1999; Brown *et al*., 2010; Divon *et al*., 2019).

1.2.5. Disease symptoms

Initial FHB disease symptom is emergence of water-soaked spots in the middle of glumes, rachis, or on the first floret and progressively propagates throughout the head everywhere the pathogen develops from the origin of infection, leading to spike drying up, which is reflected as a prematurely whitened or bleached head (partial or complete) (Teli *et al*., 2020) (Figure 1.5A and B). Under humid conditions, pathogen grows as a white or pinkish mycelia (Figure 1.5C) with pink or orange conidia masses (Figure 1.5D) on edges of the glumes of infected spikelets (Murray *et al*., 2013; Mills *et al*., 2016). Moreover, the pathogen progressively colonizes the growing grain, causing what are known as "Fusarium damaged kernels" (Figure 1.5E), which are frequently shriveled, discolored, rough-surfaced and lightweight kernels with pinkish chalky white appearance (Loughman *et al*., 2004). In the late season, small bluish-black spherical structures called as perithecia **(**Figure 1.5F**)** are produced on the surface of the damaged spikelet (Teli *et al*., 2020).

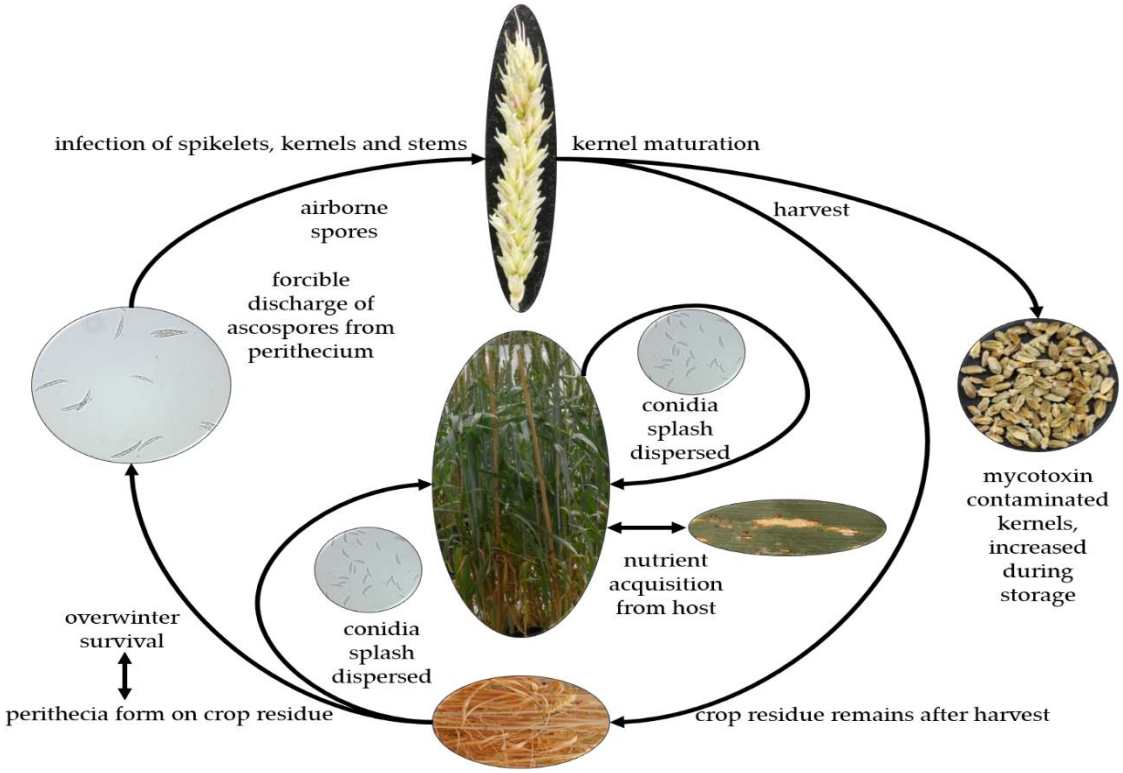


Figure 1.4. Disease cycle and symptoms of FHB on wheat spikes and kernels (Alisaac and Mahlein, 2023).

1.3. The genus *Fusarium*

Fusarium species have a widespread geographic distribution throughout the world, most frequently found in soil or plant debris, although they can also be found in water, air, plants, and insects. The main determinants of *Fusarium* distribution and pathogenic activity are climate, soil physicochemical parameters, and vegetation type (Nilsson *et al*., 2019). Currently, *Fusarium* encompasses more than 400 phylogenetically distinct species, partitioned into 23 clades referred to as species complexes (Figure 1.6) (Geiser *et al*., 2021; Torres-Cruz *et al*., 2022). Most members of the genus *Fusarium* are recognized as phytopathogens of more than 200 crop species triggering rots, wilts, blights, and cankers in agricultural and natural ecosystems, which generate multi- billion U.S. dollar losses annually to the global agricultural economy.

Some *Fusarium* species often produce harmful secondary metabolites (i.e., mycotoxins) that constitute a global threat to food and feed safety and to the health of humans and other livestock. Additionally, many Fusaria are regarded as a valuable source of a variety of bioactive secondary metabolites, including anti-cancer, antibiotics, and antioxidants compounds (Abdel-Azeem *et al*., 2019), can also produce several key enzymes for industries.

Figure 1.5. Symptoms of Fusarium head blight of wheat. A. Partial bleaching of spikelet, B. Complete bleaching of spikelet, C. Infected spikelets with white, pinkish fungal mycelia, D. Orange spores, E. Fusarium wheat seeds (on the left) and healthy wheat grains (on the right), F. Black perithecia. A and B (Scherm *et al*., 2013), C, D and F (Shude *et al*., 2020), E (Wise *et al*., 2015).

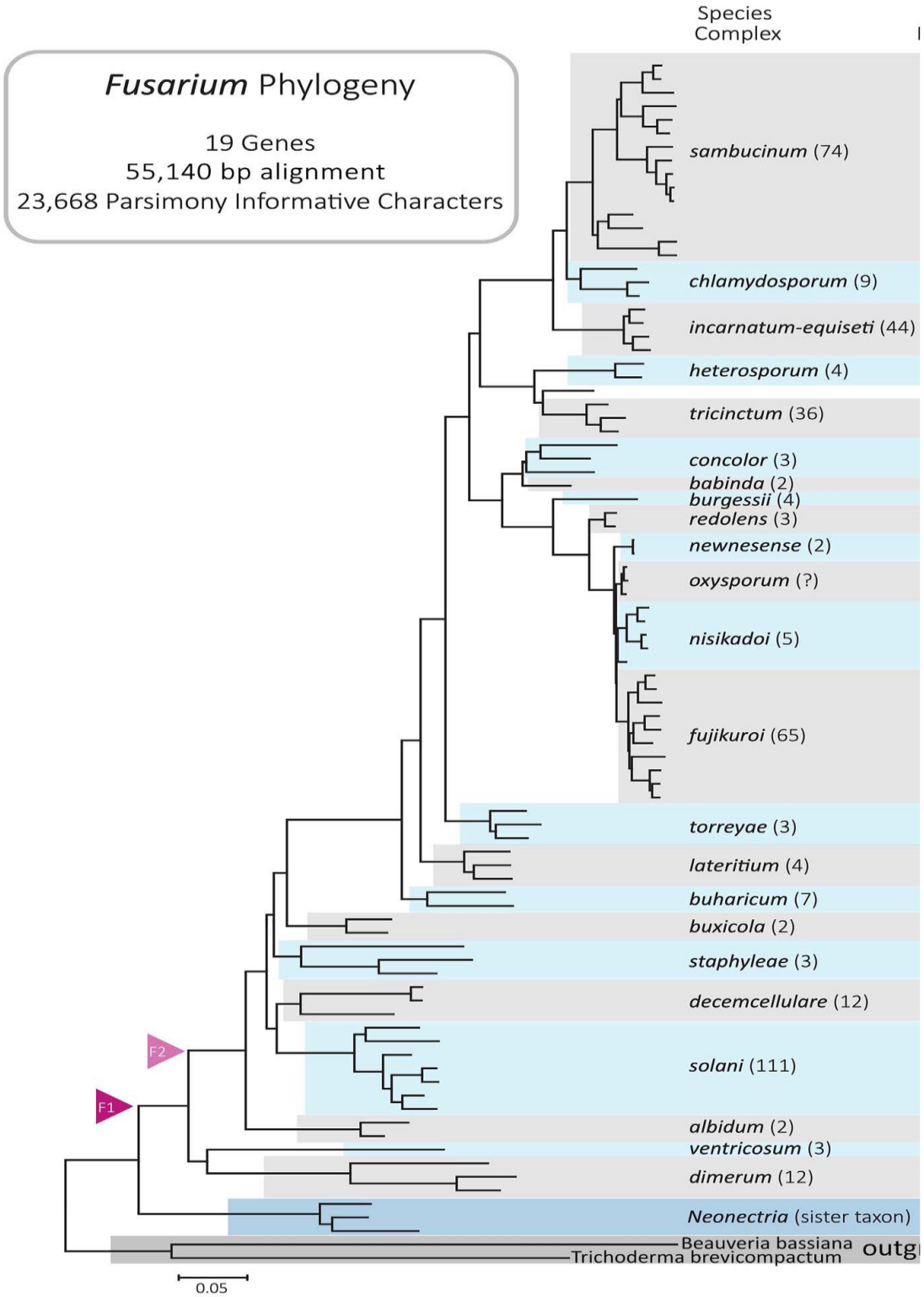


Figure 1.6. *Fusarium* phylogram inferred from exon sequences of 19 full- length protein-coding genes totalling 55.1 kb (Torres-Cruz *et al*., 2022).

1.3.1. Pathogen taxonomy

The genus *Fusarium* (from Latin *Fusus*, given the typical banana-shaped conidia) was first described by Heinrich Friedrich Link in 1809, and in1821, Fries added it to the taxonomy (Nikitin *et al*., 2023). Furthermore, in 2005, the subspecies of the genus *Microdochium* were reclassified as species and adopted as accepted taxonomy. The taxonomic positions of the genera *Fusarium* and *Microdochium* are depicted in the subsequent scheme based on the MycoBank database, 2023 (Figure 1.7) (MycoBank, 2023; Alisaac and Mahlein, 2023). The genus *Fusarium* still has a complex taxonomy, and a number of its species may exhibit quite divergent morphological, ecological, and physiological traits (Abdel-Azeem *et al*., 2019; Manganiello *et al*., 2019), while additional members of this genus, have similar morphology, metabolism, ecology, and spectrum of afflicted crops, called "species complex", e.g., the species complex of *F.incarnatum-equiseti* (FIESC).

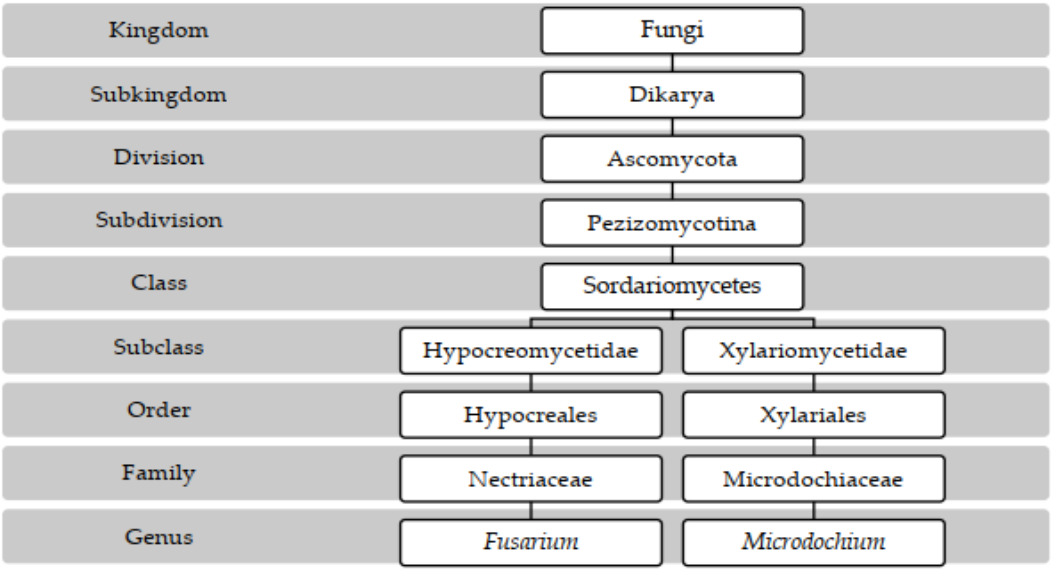


Figure 1.7. Taxonomical position of the genera *Fusarium* and *Microdochium* based on MycoBank database, 2023 (Alisaac and Mahlein, 2023).

1.3.2. Identification of *Fusarium* species

Due to the extreme heterogeneity of the genus *Fusarium* in terms of physiological, morphological, and genetic features, the identification of its species implies the combined use of many methods, especially morphological and phylogenetic. Accurate species identification of a pathogen is extremely crucial for quick and efficient diagnosis and management of illnesses and toxins production.

1.3.2.1. Morphological identification

Morphological identification of *Fusarium* species is essentially based on a combination of macroscopic and microscopic observations of several traits. Key macroscopic traits include: growth rate, presence/absence and characteristics of sporodochia and sclerotia, as well as colony characters like colony morphology, type of aerial mycelium, and pigmentation. Microscopic traits include: dimensions and characteristics of aerial conidiophores and conidiogenous cells (mono- or polyphialides), types of conidia produced, e.g., aerial and sporodochial macroconidia, aerial microconidia, as well as presence or absence, type and arrangement of chlamydospores (Leslie and Summerell, 2008).

Sporodochial macroconidia are more homogeneous in size and shape than aerial macroconidia. The overall shape, features of their apical and basal cells, and septation number are key characters in the identification of some species. The microconidia usually have 0 or 1 septations, although some species have been found to produce conidia with 2 septa, their typical shapes are: fusiform, reniform, pyriform, napiform, obovoid, globose, and oval. They can be positioned on the phialides singly, in chains, or in false heads. Chlamydospores may be formed singly, doubly, in clumps and in chains, they may be terminal or intercalary in aerial mycelia or incorporated in agar (Leslie and Summerell, 2008).

The macroconidia are the primary characteristic that distinguishes the *Fusarium* genus. However, phylogenetic analyses indicate that this morphological criterion is either still seen in other genera of ascomycetes or has disappeared in other *Fusarium* species, like *F. neocosmosporiellum* (Gräfenhan *et al*., 2011; O'Donnell *et al*., 2013). Some species are difficult to identify using phenotypic methods since their macroscopic and microscopic culture traits are fairly similar. Given this, molecular biology methods are required for the accurate identification of *Fusarium* species.

1.3.2.2. Molecular identification

Molecular genetic methods apply a series high-throughput sequencing, such as PacBio SMRT (Karlsson *et al*., 2016; Walder *et al*., 2017) and Illumina MiSeq technology (Boutigny *et al*., 2019), have been used to identify the species composition of *Fusarium* in natural substrates. The qPCR is additionally recognized as an effective tool for the detection of *Fusaium* spp. The choice of primer pairs is particularly crucial since they should specifically amplify a region of the gene within the *Fusarium* genus to properly

identify *Fusarium* species. In routine diagnosis, the following PCR primers for *Fusarium* genes amplification have proven the best outcomes: 28S large subunit of the nrDNA (LSU), ATP citrate lyase (*acl1*), Beta-tubulin (*tub2*), Calmodulin (*CaM*), Internal transcribed spacer region of the nrDNA (ITS), RNA polymerase I largest subunit 1 (*RPB1*), RNA polymerase II largest subunit 1 (*RPB2*), and translation elongation factor 1-alpha (*TEF1*-α) (Crous *et al*., 2021). *TEF1*, *RPB1*, and/or *RPB2* are the most useful for species-level identifications, given its great discriminatory potential and are well represented in databases. *TEF1* was chosen as the main *Fusarium* identification primer as it has only one copy of this gene (Geiser *et al*., 2004). Whereas the *RPB2* affords better discriminating across closely related species. On the other side, the success of PCR amplification and sequencing is frequently higher for *TEF1* than for *RPB2*. When employed for phylogenetic analysis, given that *RPB2* has a more favourably low fraction of introns compared to *TEF1*, its sequence alignments are substantially more reliable and unambiguous (Crous *et al*., 2021).

In addition to the *Fusarium* sequences found in the GenBank database, two specific *Fusarium* databases are currently accessible: *FUSARIOID-ID* (https://www. fusarium.org/ (accessed on 15 February 2023)) and *Fusarium* MLST (https://fusarium.mycobank.org (accessed on 15 February 2023)). These databases advised using the *TEF1* gene like a marker to identify *Fusarium* and sequencing the *RPB1* and *RPB2* genes to validate this identification (Geiser *et al*., 2004; O'Donnell *et al.*, 2010; Park *et al*., 2010).

Fusarium molecular biology investigations are currently primarily devoted to the analysis of full genomic sequences of the pathogen in order to identify the genes and their regulators linked to virulence and pathogenicity, primary and secondary metabolism, and potential genetic targets for the chemical control of pathogens (Summerell, 2019). Therefore, this research may disclose the complex dynamics of host-microbe interactions that cause diseases like Fusarium head blight in wheat, as well as the complexity of the genes regulating mycotoxin synthesis. This is helping to reduce mycotoxin levels in cereals, in addition to establishing strategies for breeding and fostering crops resistant to *Fusarium* and mycotoxin contamination.

1.3.3. Diversity of pathogenicity

The genus *Fusarium* has a number of virulence factors, such as mycotoxins, enzymes, and effectors. Pathogenicity genes can be distinguished into two broad classes: basic pathogenicity genes, which are common by *Fusarium* and other pathogenic fungus, and

specialized pathogenicity genes, which are often specific to particular *Fusarium* species on specific hosts (Rampersad, 2020).

Basic pathogenicity genes encode fundamental components of pathways that are responsible for detecting external or internal signals, such as those encoding different components of mitogen-activated protein kinase (MAPK) and cyclic AMP-protein kinase A (cAMP-PKA) (Ma *et al*., 2013), and mutations occurring in these genes typically impact the pathogenicity of mutants. Moreover, all *Fusarium* genomes encode a wider range of cell wall-degrading, e.g., proteases, cellulolytic enzymes, cutinase and other hydrolytic enzymes postulated to be used during infection to acquire access to nutrients. Except for the secreted lipase FGL1, which increases the virulence of *F. graminearum* on barley, wheat, and corn (Voigt *et al*., 2005; Ilgen *et al*., 2008). Additionally, the virulence of a non-pathogenic mitogen-activated kinase mutant on wheat was recovered by overexpression of the FGL1 gene (Salomon *et al*., 2012).

Diverse specialized pathogenicity genes are closely implied in host-pathogen interactions (Ma *et al*., 2013). These comprise secreted in xylem (SIX) genes (which code for small effector proteins that are secreted by *F. oxysporum* f. sp. *lycopersici* (FOL) throughout plant infection) and *Fusarium* transcription factor (FTF)-encoded genes (FTF1 and FTF2) (which are associated with the transcription of these SIX genes), whose PHI-base (http://www.phi-base.org/) describes with "reduced virulence" mutant phenotype in *F. oxysporum* in host plants *Phaseolus vulgaris* (kidney bean) (Rampersad, 2020).

Additional specialised virulence factors include mycotoxins, which are produced by some *Fusarium* species and can induce differential virulence against both wheat (*Triticum* spp.) and maize (*Zea mays*) (Proctor *et al*., 1995; Bai *et al*., 2002; Ilgen *et al*., 2008), but not barley (*Hordeum vulgare*) (Jansen *et al*., 2005). Various mycotoxin profiles can be identified in single isolates of *Fusarium* species.

Evolution of virulence through the horizontal transfer of supernumerary (SP) chromosomes between genetically isolated strains of *F. oxysporum* and *F. solani* species complexes (Coleman *et al*., 2009; Ma *et al*., 2010) led to the emergence of new pathogenic lineages.

1.4. Mycotoxins

Fusarium mycotoxins are a huge family of secondary metabolites (trichothecenes, zearalenone, fumonisins, moniliformin, beauvericin, fusarin et di acetyl scirpenol) produced by several *Fusarium* species with diverse structures and chemical composition (Table 1.2), which contaminate cereal grains, human food and animal feeds products.

Fusarium mycotoxins' fumonisins (FUMS), zearalenone (ZEA), and trichothecenes (TCT) are the most toxicologically significant classes for both human and animal health (Munkvold, 2017). It is imperative to highlight that several *Fusarium* species can produce the same toxin, and that each strain is capable of producing several toxins simultaneously. Furthermore, within the same species, the ability to produce a toxin both *in vitro* and *in vivo* fluctuates between isolates (Yli-Mattila and Gagkaeva, 2010). *Fusarium* toxins have been implicated with Fusarium head blight in various crops. As well, these mycotoxins can have adverse body effects that are either acute and/or chronic toxicities.

Table 1.2. Mycotoxins produced by *Fusarium* spp. (Gurikar *et al*., 2022).

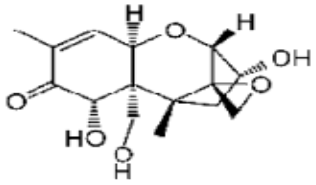
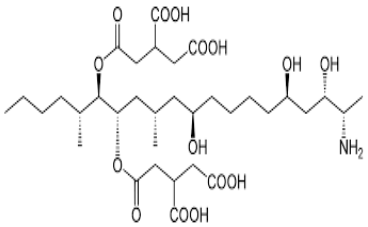
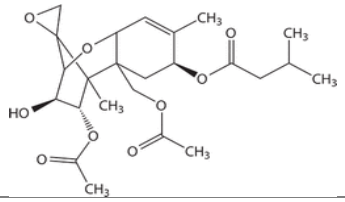
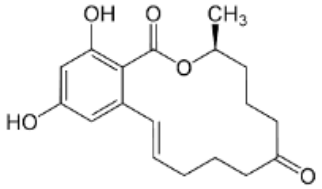
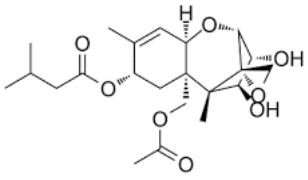
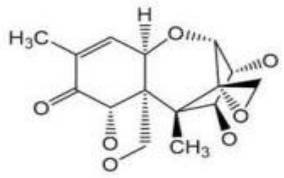
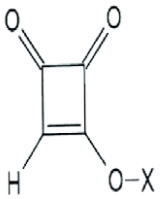
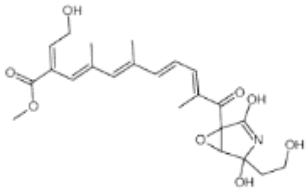
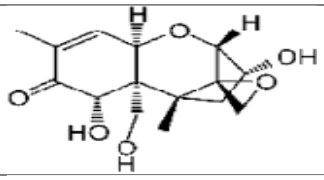
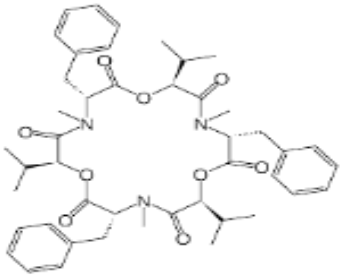
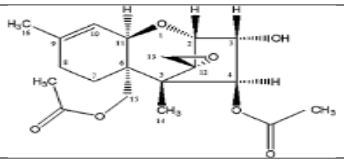
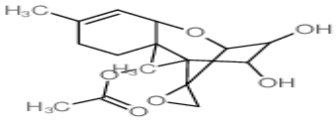
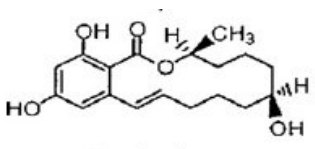
Mycotoxin	Structure of mycotoxins	Fungal specie
Deoxynivalenol		*F. graminearum*, *F. culmorum*
Fumonisins		*F. moniliforme*, *F. verticillioides*, *F. proliferatum*, *F. nygamai*
T-2		*Fusarium* species
Zearalenone		*F. graminearum*, *F. cerealis*, *F. culmorum*, *F. sambucinium*
HT-2 toxin		*F. sporotrichioides*, *F. culmorum*, *F. avenaceum*, *F. nivale*

Table 1.2. *Cont.*

Mycotoxin	Structure of mycotoxins	Fungal specie
Nivalenol		*F. cerealis*, *F. poae*, *F.culmorum*, *F. graminearum*
Moniliformin		*F. acuminatum*, *F. avenaceum*, *F.chlamydosporum*, *F. oxysporum*, *F. subglutinans*
Fusarin		*F. nivale*, *F. graminearum*, *F. oxysporum*, *F. semitectum*, *F. sporotrichioides*, *F.sambucinum*
3Acetyl-Deoxynivalenol		*F. graminearum*, *F. culmorum*
Beauvericin		*F. semitectum*, *F. subglutinans*, *F. anthophilum*, *F. avenaceum*, *F. beomiforme*, *F. dlamini*, *F. longipes*, *F. nygamai*, *F. oxysporum*, *F. sambucinum*
Di acetylscirpenol		*F. acuminatum*, *F. equeseti*, *F. sporotrichioides*
Mono acetoxyscirpenol		*F. acuminatum* *F. proliferatum*
Zearalenol		*F. cerealis*, *F. culmorum*, *F. heterosporum*

1.4.1. Trichothecenes (TCT)

Trichothecenes (TCT) are a vast family (over 200 different types of trichothecenes identified currently) of fungal secondary metabolites produced primarily, but not exclusively, by *Fusarium* species (24 different *Fusarium* species). Further TCT producing genera encompass *Trichoderma*, *Verticimonosporium*, *Trichothecium*, *Mycothecium*, *Cephalosporium*, and *Stachybotrys* (Wu *et al*., 2017). They have been encountered to mostly contaminate cereal crops (such as wheat, barley, maize, oats, and rye) globally.

1.4.1.1. Chemical structure

Trichothecenes are sesquiterpenoid compounds belong to a class of terpenes with three isoprene units, and are composed of 9,10 double bonds and 12,13 epoxyalkylene groups that are cytotoxic (McCormick *et al*., 2011) (Figure 1.8). They were classified into four types (A, B, C, and D) according to their functional groups and fungus producing them, of which type A (TCTA) and/or B classes (TCTB) are produced by *Fusarium* species and are the most toxic (Shank *et al*., 2011; Wu *et al*., 2011). They differ from each other by substituting in five positions along the structure (C_3, C_4, C_7, C_8, and C_{15}). Hydrogen (-H), hydroxyl (-OH), ester-linked acetyl (-OC(=O) CH_3), or ester-linked isovalerate (-OC(=O)$CH_2CH(CH_3)_2$) groups are frequent substitutes for *Fusarium* trichothecenes (Foroud *et al*., 2019). The main *Fusarium* trichothecenes and their functional groups are shown in Table 1.3.

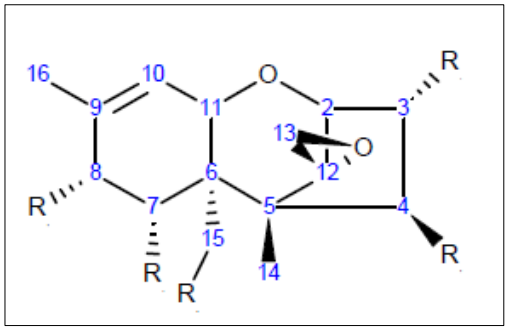


Figure 1.8. Backbone structure of trichothecene toxins (Foroud *et al*., 2019).

Table 1.3. Substituent patterns of different type A and B trichothecenes (Foroud *et al*., 2019).

	C_3	C_4	C_7	C_8	C_{15}
Type A					
diacetoxyscirpenol (DAS)	-OH	-OAc	-H	-H	-OAc
trichodermin	-H	-OAc	-H	-H	-H
trichodermol	-H	-OH	-H	-H	-H
T-2 toxin	-OH	-OAc	-H	-OIsoval	-OAc
HT-2 toxin	-OH	-OH	-H	-OIsoval	-OAc
NX-2	-OAc	-H	-OH	-H	-OH
NX-3	-OH	-H	-OH	-H	-OH
Type B					
nivalenol (NIV)	-OH	-OH	-OH	=O	-OH
4-*O*-acetyl-NIV (4ANIV)	-OH	-OAc	-OH	=O	-OH
4-deoxy-nivalenol (DON)	-OH	-H	-OH	=O	-OH
3-*O*-acetyl-DON (3-ADON)	-OAc	-H	-OH	=O	-OH
15-*O*-acetyl-DON (15-ADON)	-OH	-H	-OH	=O	-OAc
trichothecin	-H	-OIsoval	-H	=O	-H

OAc = O-acetyl, OIsoval = O-isovalerate.

A ketone (=O) at C8 differentiates TCTB from TCTA. More recently, a novel TCTA called NX-2 and its derivatives (NX-3 and NX-4) were detected in *F. graminearum* (Varga *et al*., 2015). These emerging mycotoxins share structural similarities with the TCTB 4-deoxynivalenol (DON) and 3-O-acteyl-DON (3-ADON), respectively, just the C8 ketone's presence distinguishing them. Within type B, two chemotypes have been identified, chemotype I producing DON and its two acetylated derivatives (3-ADON and 15-ADON), and chemotype II producing NIV and/or 4 acetylnivalenol (4ANIV) (Mielniczuk and Skwaryło-Bednarz, 2020).

1.4.1.2.Trichothecene biosynthesis pathway

Biosynthesis of *Fusarium* trichothecenes is carried out by *TRI* cluster of 15 genes which located at three different loci on different chromosomes: the "*TRI5* cluster" locus includes 12 *TRI* genes, the two genes *TRI1- TRI16* locus and the single gene *TRI101* locus (Figure 1.10). The cluster includes also further genes *TRI6* and *TRI10* that encode regulatory proteins, the *TRI12* transporter and unidentified proteins (Kimura *et al*., 2007; Alexander *et al*., 2009). The first biosynthetic step involves trichodiene synthase encoded by the *TRI5* gene in the cyclization of farnesyl pyrophosphate to form trichodiene (Nozoe and Machida, 1970; Hohn and Beremand, 1989). This step is followed by a series of cyclization, isomerization, oxygenation, and acetylation reactions and finishes by the formation of one of the TCT (Figure 1.9) (Kimura *et al*., 2007; Alexander *et al*., 2009; McCormick *et al*.,

2011). The production of DON and NIV chemotypes is controlled by *TRI* cluster genes *TRI13* and *TRI7* (Lee *et al*., 2002). *TRI7* and *TRI13* genes activity defines isolates with the NIV chemotype, whereas their inactivity results in isolates with the DON chemotype. The *TRI8* gene controls the biosynthesis of 3-ADON and 15-ADON.

1.4.2. Fumonisins (FUMS)

Fumonisins (FUMS) are the most significant mycotoxins in contaminated maize and its products, though reports of their occurrence in a diverse range of cereals and other important crops have also been recorded (Scott, 2012). This toxin is produced by *Fusarium verticillioides, Fusarium proliferatum, Fusarium sacchari, Fusarium subglutinans, Fusarium fujikuroi,* and several other species (Perincherry *et al.*, 2019). Contrarily, fumonisin B2 (FB2) production by *Aspergillus niger* has been revealed (Frisvad *et al.*, 2007). They are recognized to be somewhat heat stable and are slightly degraded by food processing techniques.

1.4.2.1. Chemical structure

FUMS have a relatively simple chemical structure similar to that of sphingosine, which characterized by a long chain (20 carbon atoms) of polyhydroxy alkylamines with two propane tricarboxylic acid moieties (tricarballylic acid, TCA) that are esterified to hydroxyl groups on adjoining carbon atoms (Ocampo-Acuna *et al.*, 2023). To date, 28 distinct structures of FUMS have been identified, which are categorized into the following four series based on their chemical structure (Figure 1.11): series-A refers to amides, series-B includes a free amine group and a terminal methyl, series-C corresponds to a terminal amine group, and series-P integrated a 3-hydroxypiridinium residue in their structures (Yazar and Omurtag, 2008; Braun and Wink, 2018).

Fumonisins B (FB1, FB2, FB4 and FB5) are the most pertinent given their prevalence on many foodstuffs and crops. FB1 is the most abundant and most toxic FBs.

1.4.2.2. Fumonisins biosynthesis pathway

The biosynthesis pathway for FUMS, which are polyketides, is controlled by the *FUM* gene cluster (Figure 1.12). In the first step of FUMS biosynthesis, a full straight chain of 18 carbons with methyl groups at C12 and C16 is formed by a polyketide synthase (PKS) (Kim *et al.*, 2020). Second, the polyketide is condensed with alanine by an aminotransferase (AT), forming a linear 20-carbon chain containing the two methyl groups, an amine group at C2, and a keto group at C3 (Proctor *et al.*, 2008). Third, the C3

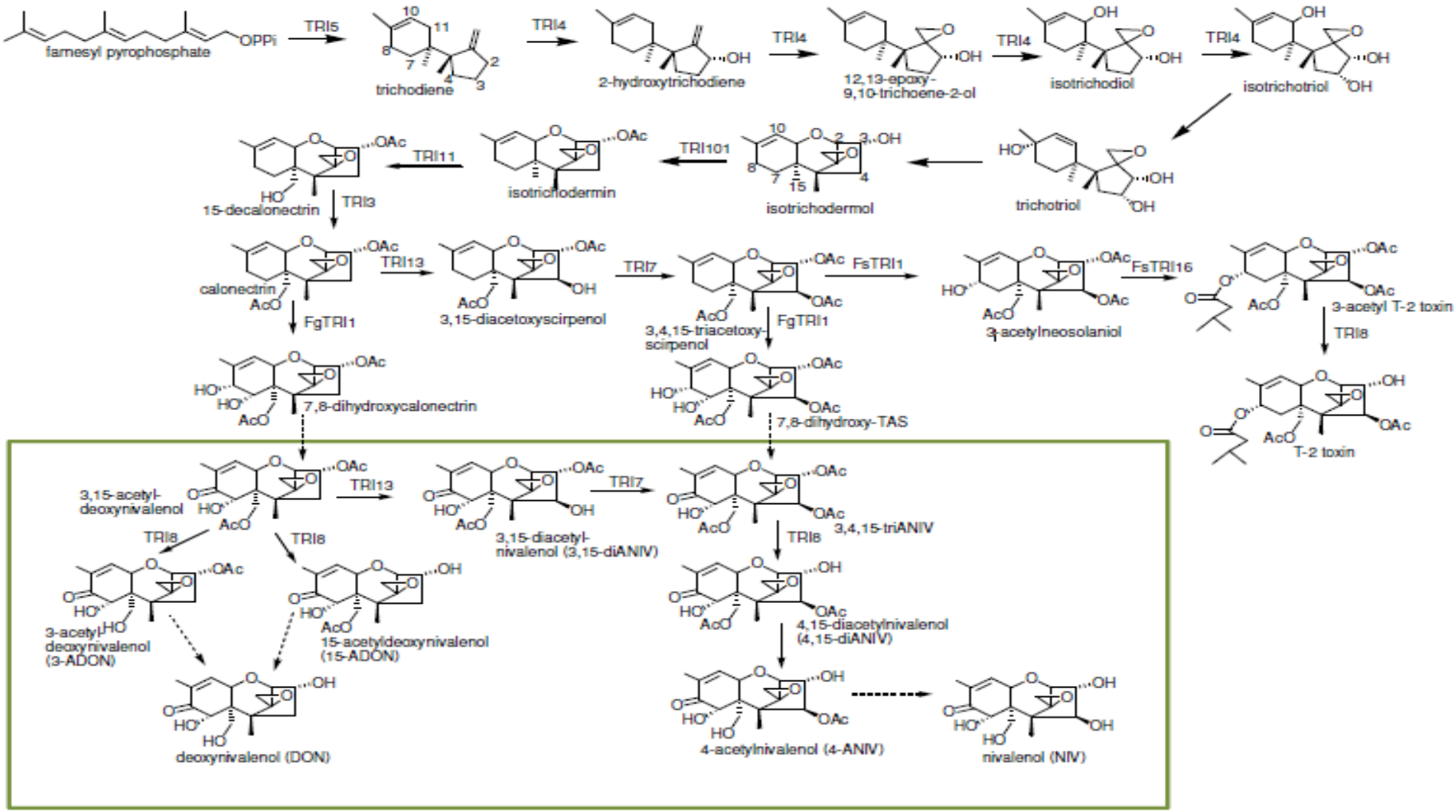


Figure 1.9. Trichothecene biosynthetic pathway (McCormick *et al*., 2011).

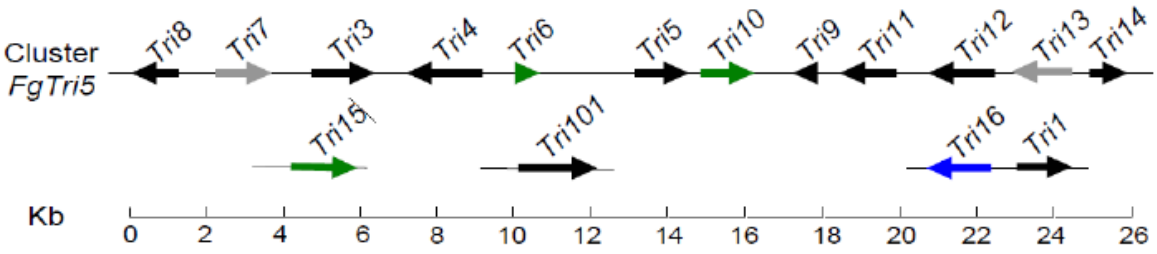


Figure 1.10. Schematic representation of the *TRI* cluster and the two loci grouping the *TRI* genes in *F. graminearum*. *Tri8*: trichothecene-3-O-esterase, *Tri7*: trichothecene-4-O-acetyltransferase, *Tri3*: trichothecene-15-O-acetyltransferase, *Tri4*: trichodiene oxygenase, *Tri6*: transcription factor, *Tri5*: trichodiene synthase, *Tri10*: regulatory gene, *Tri9*: unknown, *Tri11*: isotrichodermin 15-oxygenase, *Tri12*: trichothecene membrane transporter, *Tri13*: calonectrin 4-oxygenase, *Tri14*: virulence factor, *Tri1*: C-8 or C-7,8 oxygenase, *Tri16*: C-8 acetyltransferase, *Tri101*: C-3 acetyltransferase, *Tri15*: regulatory gene (Merhej *et al.*, 2011 ; Alexander *et al.*, 2009).

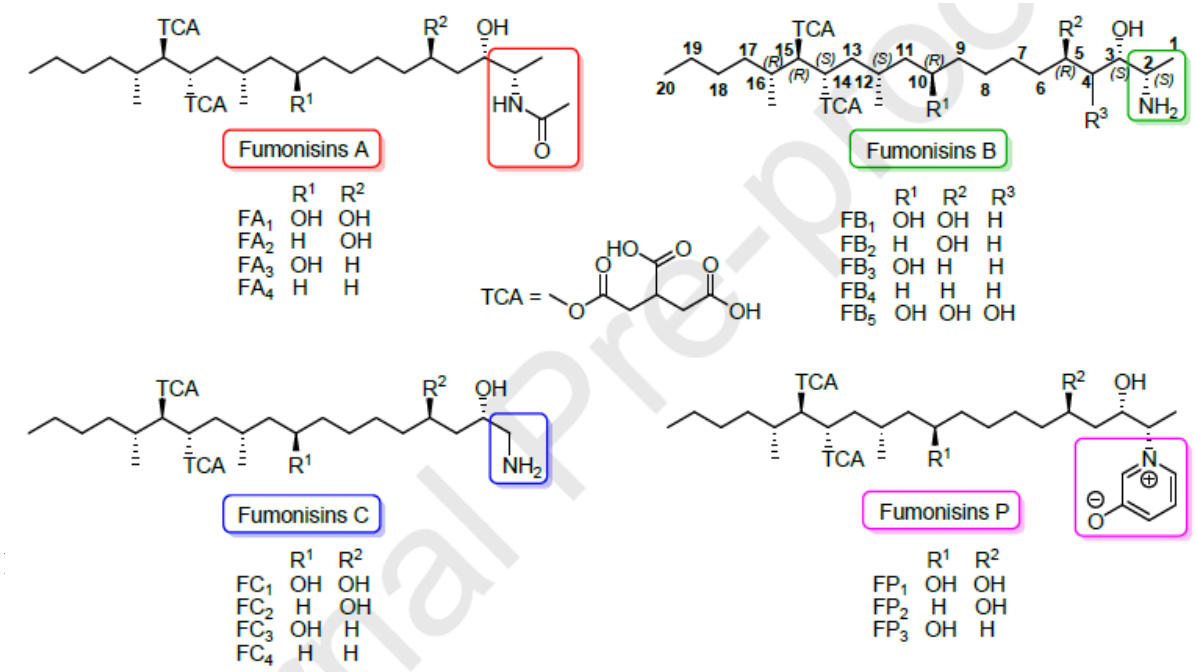


Figure 1.11. Chemical structures of fumonisins (Ocampo-Acuna *et al.*, 2023).

keto group is reduced to a hydroxyl by a short-chain dehydrogenase reductase (SDR) (Butchko *et al.*, 2003). FB1, FB2, FB3, and FB4 are derived from subsequent hydroxylation of the polyketide backbone at positions C4, C5, C10, C14, and C15, as well as esterification of tricarboxylate molecules to the hydroxyls at C14 and C15 (Alexander *et al.*, 2009). The structural differences between fumonisins C (FCs) and FBs relate to the presence (FBs) or absence (FCs) of a terminal methyl group adjacent to the amine. This structural difference is due to the condensation of the precursor polyketide with two various amino acids: alanine in FB biosynthesis and glycine in FC biosynthesis (Branham and Plattner, 1993; Proctor *et al.*, 2008). The choice of amino acid used in biosynthesis is defined by the specificity of the AT (Fum8) in FB versus FC-producing species' amino acid substrates (Proctor *et al.*, 2008).

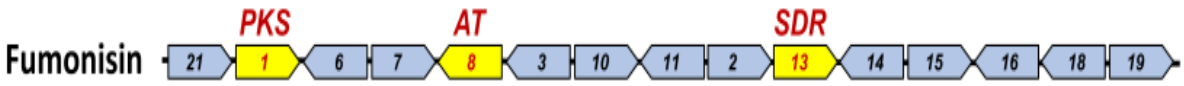


Figure 1.12. Organization of genes in *FUM* gene cluster. The numbers in the arrows represent the number of the *FUM* gene (e.g., 21 indicates the *FUM21* gene) (Kim *et al.*, 2020).

1.4.3. Zearalenone (ZEA)

Zearalenone (ZEA), previously known as F-2 toxin, is a non-steroidal estrogenic mycotoxin frequently contaminates maize but can also affect other cereal crops worldwide (Figure 1.13). Occurrence of ZEA has also been reported in food of plant and animal (Bai *et al.*, 2018). Toxin production by a number of *Fusarium* species, include *F. graminearum, F. culmorum, F. cerealis* (syn. *F. crookwellense*), *F. equiseti* and *F. semitectum* has mostly been described (Nahle *et al.*, 2021). ZEA is thermostable and is not degraded during storage, heating, or milling processing.

1.4.3.1. Chemical structure

ZEA is part of the xenoestrogens, has the general formula $C_{18}H_{22}O_5$ and is a 6-(10-hydroxy-6-oxy-trans-1-undecenyl-beta-resorcylic acid lactone) (Urry *et al.*, 1966), which exhibits similarities with natural estrogens *in Animalia*, like 7β-estradiol and can therefore link to estrogen receptors exerting its hormonal action which is superior to any other natural non-steroidal estrogen. The structures of ZEA and its derivatives are illustrated in (Figure 1.14).

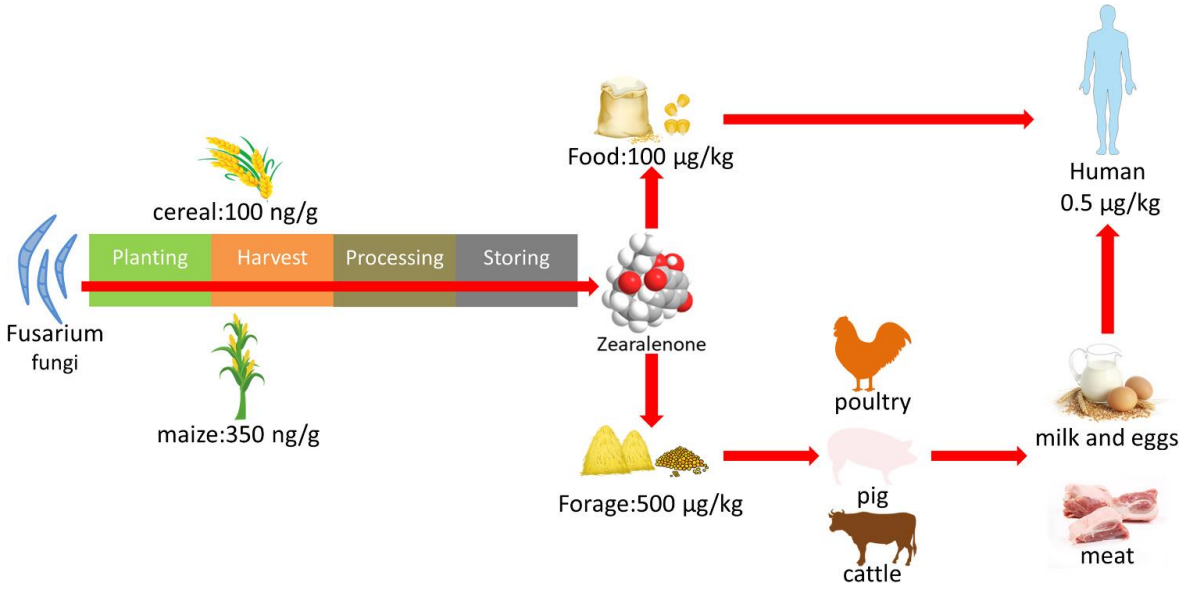
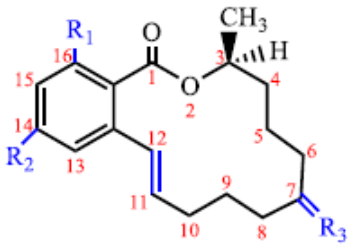


Figure 1.13. Schematic of ZEA contamination pathways (Li *et al*., 2021).



Compounds	R1	R2	R3	C_{11}-C_{12} double bone
ZEN	OH	OH	O	trans
ZAN	OH	OH	O	dihydro
α-ZEL	OH	OH	H, α-OH	trans
β-ZEL	OH	OH	H, β-OH	trans
α-ZAL	OH	OH	H, α-OH	dihydro
β-ZAL	OH	OH	H, β-OH	dihydro
Z14G	OH	$C_6H_{11}O_6$	O	trans
Z16G	$C_6H_{11}O_6$	OH	O	trans
Z14S	OH	SO_4^-	O	trans
Z14-phosphate	OH	H_2PO_4	O	dihydro
α-ZEL-G	OH	$C_6H_{11}O_6$	OH	trans
β-ZEL-G	OH	$C_6H_{11}O_6$	OH	trans
6'-acetyl-β-ZAL	OH	OH	H, β-OAc	dihydro
6'-acetyl-β-ZEL	OH	OH	H, β-OAc	trans

Figure 1.14. Structures of ZEA and its derivatives (Lu *et al*., 2022).

1.4.3.2. Zearalenone biosynthesis pathway

ZEA is biosynthesized by the polyketide pathway, which is controlled by the ZEA gene cluster (Figure 1.15). The majority of clusters related to polyketide biosynthesis include one PKS gene in addition to genes encoding modifying enzymes involved in the hydroxylation, oxygenation, halogenation, alkylation, and cyclization steps that transform the polyketide backbone into a final polyketide metabolite (Hertweck, 2009). Regulatory protein-encoding genes may also be present in the cluster. Understanding the mechanism of ZEA biosynthesis has been simplified by the detection of the two PKS genes (PKS13 and PKS4) in the ZEA cluster (Nahle *et al*., 2021). ZEA biosynthesis is initiated from a single molecule of acetyl-CoA and eight molecules of malonyl-CoA (Gaffoor and Trail, 2006) (Figure 1.16). The PKS4 synthesizes and reduces the first 10 carbon additions, releasing this portion to be taken as a precursor by the nonreducing PKS13, which fulfills the carbon additions for the ZEA backbone. The part of the molecule that contains the unreduced ketones is extremely reactive and rapidly aromatizes. ß-Zearalenol is liberated from PKS13 via macrolactonization following the completion of the backbone's synthesis, and afterwards oxidized by a putative isoamyl alcohol oxidase (ZEB1) to ZEA molecule (Figure 1.16).

1.4.4. Emerging *Fusarium* toxins

New mycotoxins, known as "emerging mycotoxins", which are described as chemical substances whose occurrence was extensively encountered in raw cereals as well as other food and feed commodities, and are therefore becoming a public health and economic challenge. In contrast to strictly regulated mycotoxins, including DON, ZEA, FUMS, and T-2 and HT-2 toxins (T-2 and HT-2), emerging mycotoxins are not strictly regulated in legislation at present. These emerging mycotoxins usually include enniantins (ENNs), beauvericin (BEA), fusaproliferin (FUS), moniliformin (MON), and fusarin C.

- **Enniatins (ENNs),** are cyclohexadepsipeptides that alternately contain three N-methyl amino acids and three hydroxyl acids residues in their structure (Ekwomadu *et al*., 2021). They are lipophilic molecules, inhibitors of cellular membrane transport proteins and particularly toxic to mitochondria. ENNs show antifungal, antibacterial, and insecticidal activities, as well as potential herbicidal activities (Ekwomadu *et al*., 2021).

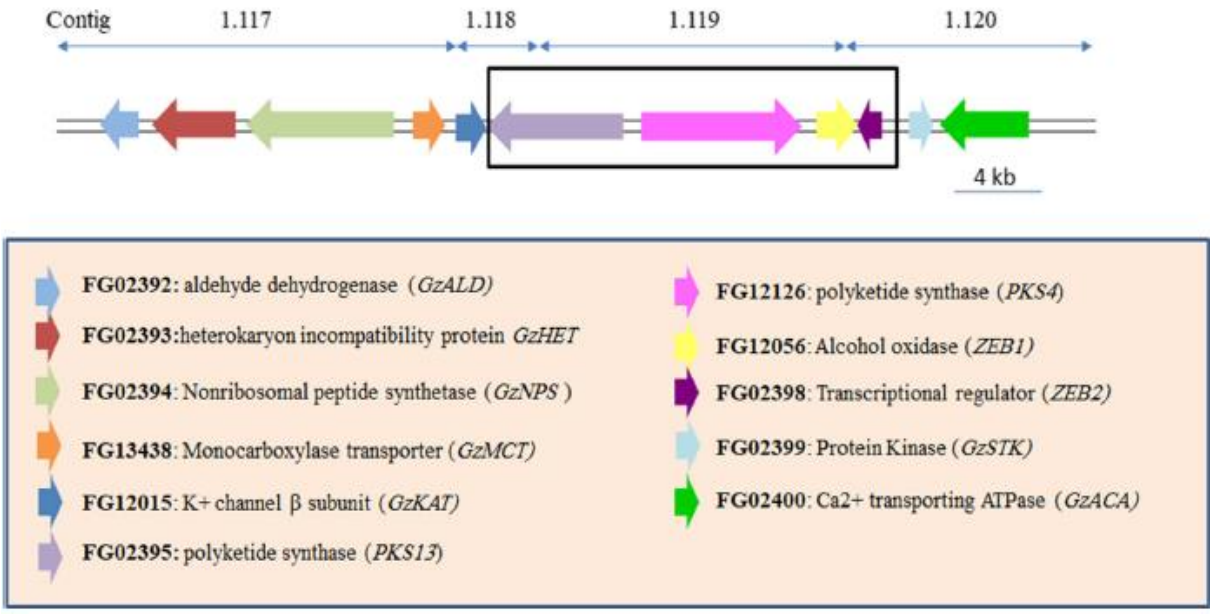


Figure 1.15. Genomic organization of the ZEA gene cluster and flanking region in *F. graminearum*. The arrows show the estimated position and direction of each gene's or ORF's transcription. Gene name is written adjacent to each arrow. The box includes genes responsible for the biosynthesis of ZEA in *F. graminearum*. The thick bar with the contig number above represents each DNA region of contigs in the *F. graminearum* genome databases (Nahle *et al*., 2021).

- **Beauvericin (BEA)**, is a cyclic hexadepsipeptide that is produced by several toxigenic fungi. It exhibits significant antibacterial activity against a variety of bacteria. Normally, it inhibits cholesterol acyltransferase (Jajić *et al*., 2019). BEA often alters cellular membrane permeability and perturbs cell homeostasis (Ekwomadu *et al*., 2021).
- **Moniliformin (MON),** is an organic acid that naturally occurs as a sodium or potassium salt and has the chemical formula 3-hydroxycyclobut-3-ene-1,2-dione (Ekwomadu *et al*., 2021). It is produced by several *Fusarium* species, primarily by *Fusarium proliferatum* and typically contaminates a variety of cereal crops, including wheat, barley, maize, oats, rice, and rye. Intake of MON has been linked to the emergence of several diseases in humans, such as Kashin-Beck and Keshan diseases.
- **Fusaproliferin (FUS),** is a bicyclic sesterterpene with five isoprenic units, found from maize cultures of *F. proliferatum* (Ekwomadu *et al*., 2021). It has been proven to be toxic to human B lymphocytes and to a number of insect cell lines (Jestoi, 2008).
- **Furasin C,** produced by *F. verticilioides*, *F. tricinctum*, or *F. graminearum*. It is mutagenic for bacteria (positive Ames test) (Heit, 2015).

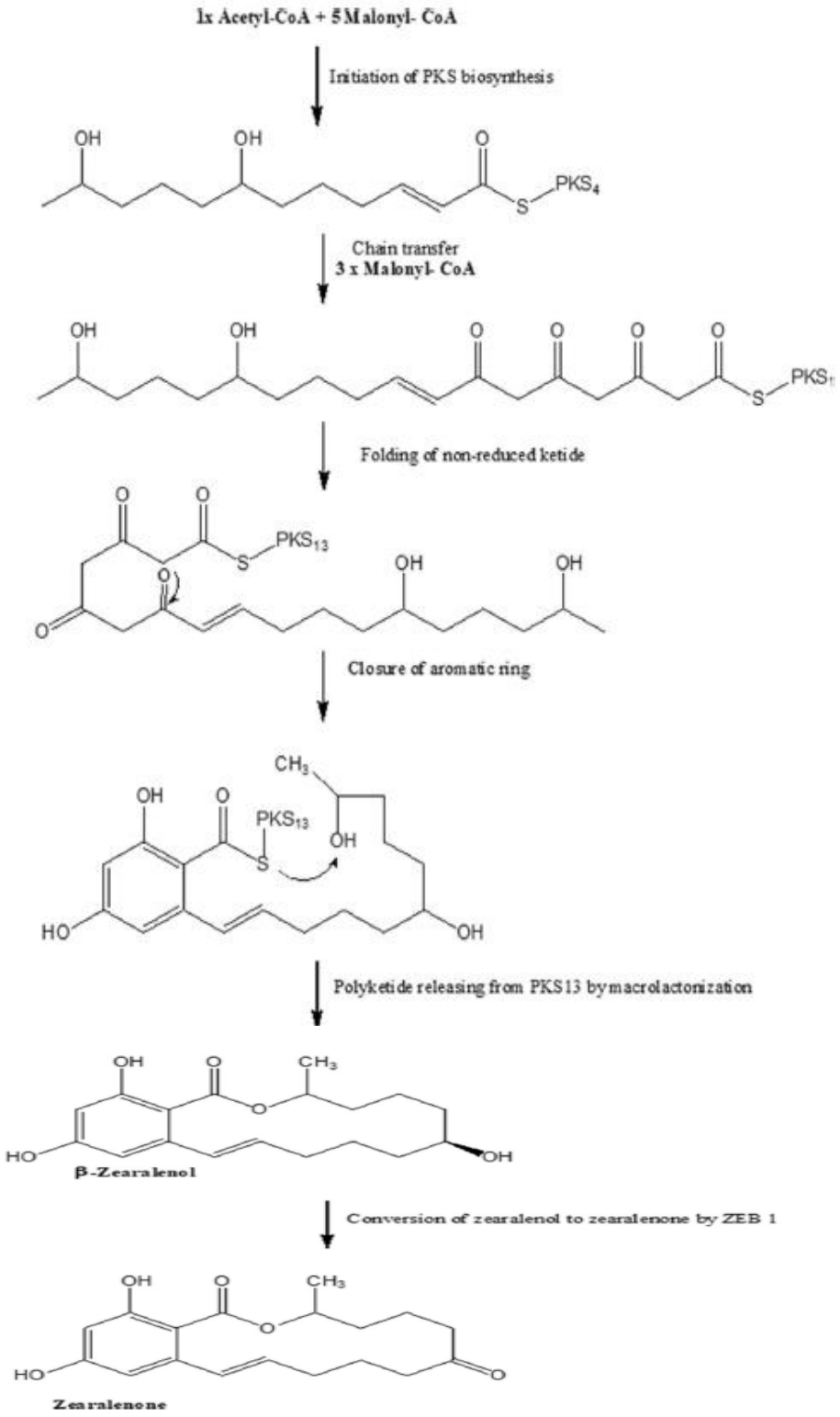


Figure 1.16. Zearalenone biosynthesis pathway (Nahle *et al*., 2021).

1.4.5. Analysis of *Fusarium* mycotoxins

In order to determine the concentration of these health hazards in various samples, it is worthy to use accurate and reliable analytical tools that enable their identification and precise quantification at trace levels (Figure 1.17). These are needed for continuous monitoring and management of potential mycotoxin risks in cereals and its products for the safeguard of consumers. These approaches are mainly divided as instrumental and bioanalytical procedures, which are chosen based on the identification requirements.

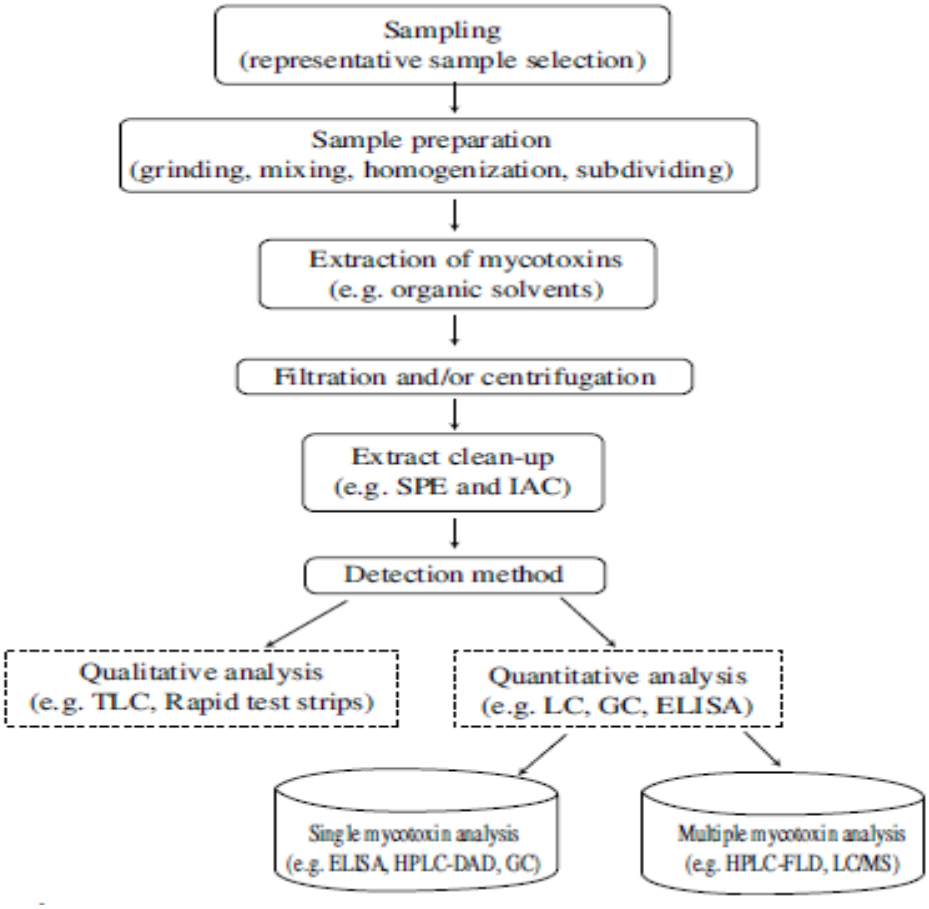


Figure 1.17. Flow diagram of common steps involved in mycotoxins analysis in food commodities (Alshannaq and Yu, 2017).

- **Chromatographic methods**

  Mycotoxins can be detected using a variety of instrumental methods, including: thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) coupled with different detectors (e.g., ultraviolet (UV) detection, mass spectrometric (MS) detection). Gas chromatography can couple with mass spectrometry (MS) detection. These approaches are applicable to both quantitative and qualitative studies,

provide excellent precision and accuracy. Noteworthy, such methods also act as reference techniques to validate immunochemical assays.

- **Immunochemical methods**

  Immunoassays focused on antibody-antigen reactions are simple and have been adopted for quick mycotoxin detection. Among them, enzyme linked immunosorbent assays, chemiluminescence immunoassays, fluorescence immunoassays, time-resolved immunochromatographic assays, fluorescence resonance energy transfer immunoassays, enzyme-linked aptamer assays, and metal-enhanced fluorescence assays (Chauhan *et al*., 2016). Immunochemical techniques provide higher selectivity for monitoring mycotoxin concentrations than chromatographic techniques, which is crucial for ensuring food safety.

1.4.6. Regulation and legislation

Regulations on mycotoxins have been established in many countries owing to the threats to human and animal health resulting from their occurrence in food and feed items. In Europe, regarding the occurrence of several mycotoxins in cereals and its derivatives, the European Commission enacted regulation 1881/2006 (Table 1.4). Up to now, Algeria has not set a maximum level for *Fusarium* mycotoxins allowed in cereal (local or imported), food, and feed. In fact, a major portion of cereal traded in Algeria is imported, so little is known of their contamination by toxins (Mahdjoubi *et al*., 2020).

1.5. Control strategies of FHB

The following methods of control are requested in practice:

1.5.1. Chemical control

Nowadays, the main method of mitigating FHB and mycotoxins contamination remains the use of fungicides, particularly DMIs (Figure 1.18) at anthesis stage. The hurdles to treatment with these fungicides are the rapid emergence of resistant strains and unsuitable climatic conditions (Chen *et al*., 2022). Consequently, albeit the more effective fungicides cannot completely prevent FHB disease. The following parameters affecting the effectiveness fungicide should be taken into account: (1) the favourable climate; (2) FHB-resistant cultivars; (3) fungicide type and dose; (4) application frequency and timing; and (5) yield gain (Dweba *et al*., 2017). Moreover, research conducted in the United States has revealed that spring wheat has higher fungicide effectiveness than soft winter wheat (Paul *et al*., 2008). There are further approaches for managing *Fusarium* infections, including:

Table 1.4. Allowable limits of *Fusarium* mycotoxins in cereals and its derivatives (European Commission, 2006, 2013).

Toxin	Applicable Products	Limit ($\mu g.kg^{-1}$)
ZEA	Processed cereals for infants and young children	20
	Bread and breakfast cereals	50
	Grain products that can be eaten directly	75
	Corn, corn snacks, and corn breakfast cereals that can be eaten directly	100
	Corn flakes larger than 500 μm in size	200
	Corn flakes less than or equal to 500 μm in size	300
	Corn treated via wet grinding	350
	Refined corn oil	400
FUMS	Corn-based baby foods	200
	Corn snacks and corn breakfast cereals	800
	Corn, corn snacks, and corn breakfast cereals that can be eaten directly	1000
	Corn flakes larger than 500 μm in size	1400
	Corn flakes less than or equal to 500 μm in size	2000
	Corn treated via wet grinding	4000
T-2 + HT-2	Unprocessed barley and maize	200
	Unprocessed oats	1000
	Unprocessed wheat, rye and other cereals	100
DON	Raw durum and oats, wet-milled corn	1750
	Unprocessed cereals other than hard wheat, oats, and corn	1250
	Cereal that can be consumed directly and cornflakes less than or equal to 500 μm in size	750
	Bread, snacks, desserts, and breakfast cereals	500
	Cereal-based foods for infants and young children	200

(1) the application of fungicide mixtures with various active ingredients (Buchneva *et al*., 2019); (2) the synthesis of innovative and efficient chemicals; (3) fungicides can be included under an integrated management program in combination with other control strategies. Triazole or benzimidazole and their combinations are the most effective fungicides for the control of FHB in wheat (Belabed *et al*., 2022; Francesconi *et al*., 2023). In China, the FHB was controlled with the fungicide phenamacril (experimental code JS399-19), which is particular to the *Fusarium* (Tang *et al*., 2018). More recently, a new succinate dehydrogenase inhibitor called pydiflumetofen (ADEPIDYN^{TM}), which was invented by Syngenta Co. (China), has been approved for the treatment of FHB in China, USA, and a number of other countries (Chen *et al*., 2022). In Algeria, the triazoles (triadimenol, tebuconazole, difenoconazole, prothioconazole and cyproconazole), as well as spiroketalamines, oximino acetates, methoxy acrylate, phenylpyrroles, are the most commonly used fungicides for FHB control (Appendix 1) (DPPTC, 2017). Further new formulations are also registered such as: Mystic 430, Nazole, Phytazox 25% Sc, and Tebuphyt 250 g.L^{-1} (Appendix 1) (DPPTC, 2021). Noteworthy, the efficiency of the active ingredients fluctuates between *Fusarium* species. The applying of fungicide-treated seed is a very effective and useful strategy for avoiding crown rot in the initial phases of crop production (Moya-Elizondo and Jacobsen, 2016).

1.5.2. Cultural control

The primary source of inoculum is cropping residues. Therefore, the ideal control strategies focused on decreasing the initial inoculum level in the soil and/or decreasing its effectiveness (Jiménez Diaz, 2011). Some of the most important cultural practices include: tillage, crop rotation, appropriate use of fertilizers, fallow, proper irrigation system, and weed control. Intercropping cereals with non-*Fusarium* host plants is a better approach to lower the prevalence of FHB in various agricultural systems (Shah L *et al.,* 2018; Xia *et al.,* 2020). The rotation is still somewhat unsuccessful since some *Fusarium* species, such as *F. culmorum* and *F. pseudograminearum*, have longevity chlamydospores (Cook, 2010). Crop rotation and extensive tillage promote pathogen suppression, Contrary to long-term monoculture and no-till farming, which subsequently results in the accumulation of phytopathogenic microorganisms (Chandrashekara *et al*., 2012). The application of organic fertilizers enhances the overall soil suppressiveness to diseases compared to mineral fertilization systems (Semenov *et al*., 2022). The introduction of several distinct taxa of arbuscular mycorrhizal fungus into the soil may be the best strategy to improve plant

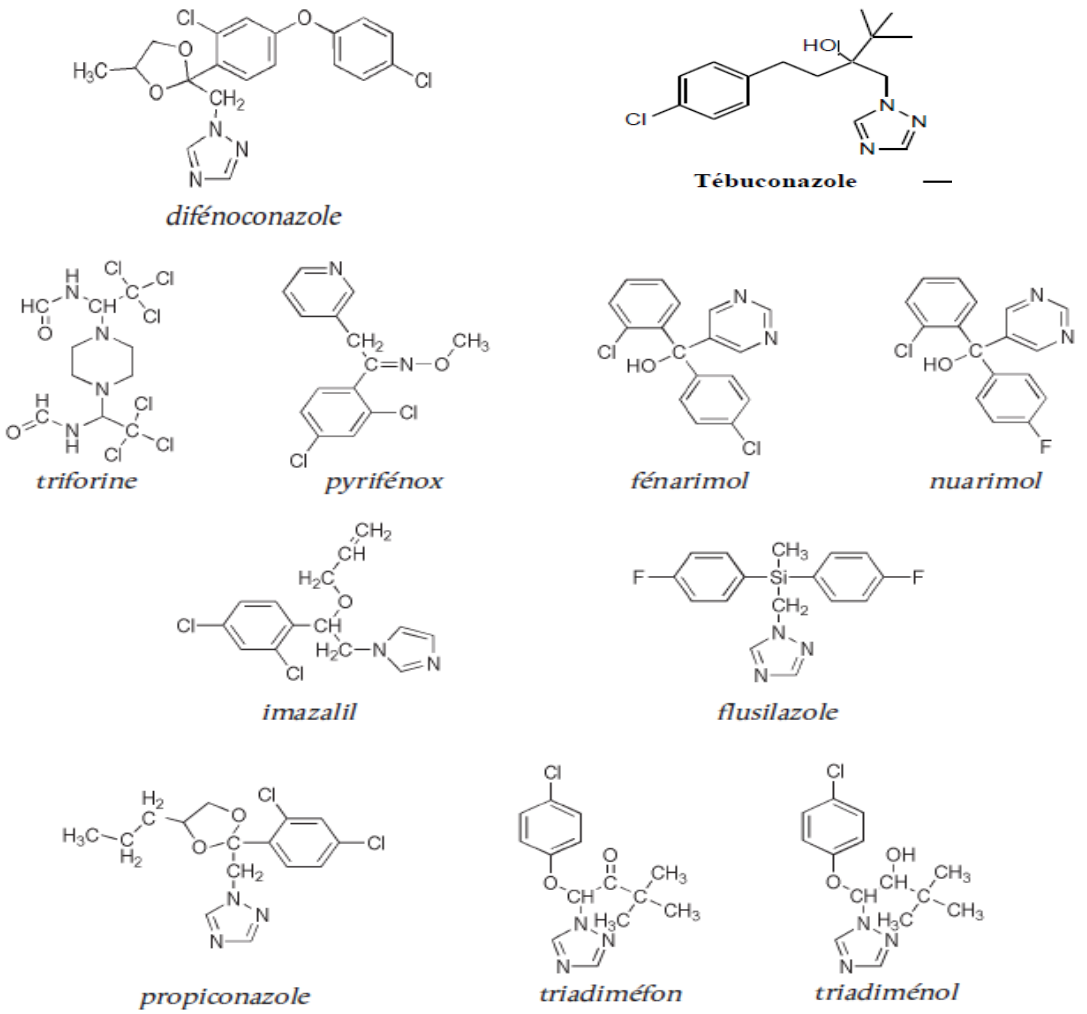


Figure 1.18. Structures of inhibitors of 14α-demethylase (IDM) (Rocher, 2004; Youness, 2013).

resistance to phytopathogens by boosting phosphorus and nitrogen feeding, plants' non-specific resistance to microorganisms (Jain *et al*., 2019). *Glomus intraradices* produces an unidentified antimicrobial compound that serves to control the conidial germination of *Fusarium oxysporum* (Filion *et al*., 1999).

1.5.3. Biological control

In light of the emergence of the resistance of the pathogens to pesticides, biological control has been adopted as an alternative to chemicals, which focuses on the introduction of populations of antagonistic microorganisms. Hence, the use of biocontrol agents is considered an eco-friendly approach. Biological control agents (BCAs) can be used singly or in combination with other control strategies under an integrated management program (IMP). There are now commercially available biopesticides and biofertilizers based on

Pythium, Pseudomonas, and Trichoderma species (Dendouga *et al*., 2016; Palazzini *et al*., 2016; Comby *et al*., 2017). Moreover, a variety of natural fungicides composed of plant extracts, including phenolic compounds and plant-extracted essential oils have been proposed as potential alternatives to synthetic fungicides (Ferrigo *et al*., 2016; Shah L *et al.,* 2018). The establishment an adequate formulation and application is the fundamental challenge to their effective usage (Legrand *et al*., 2017). Many fungal, bacterial, mycoviruses, and yeast strains can significantly minimize FHB severity and/or mycotoxins levels in contaminated grains. Several bacteria as antagonists like strains of *Streptomyces* spp*., Pseudomonas* spp*., Bacillus* spp*.,* and *Lactobacillus plantarum* have been studied against *F. graminearum* (Shude *et al*., 2020), and their impacts are typically caused by a multitude of biological control mechanisms, including competition for nutrients, mycoparasitism, and antibiosis against *F. graminearum* (Legrand *et al*., 2017). Fungal BCA antagonists against FHB include *Trichoderma* spp., *Microsphaeropsis* spp., and *Clonostachys rosea* strain ACM941 (Bujold *et al*., 2001; Hue *et al*., 2009; Matarese *et al*., 2012). Only a few yeast strains from the genera *Cryptococcus, Sporobolomyces,* and *Rhodotorula* were also tested against *F. graminearum* and *F. culmorum* (Khan *et al*., 2004; Schisler *et al*., 2014; Legrand *et al*., 2017). A number of mycoviruses used as biocontrol agents such as: *Fusarium graminearum* mycotymovirus1 (FgMTV1/SX64) and *Fusarium boothi* large flexivirus 1 (FbLFV1) have been detected from *F. graminearum* strains SX64 and *Fusarium boothi* respectively and they decrease the virulence of their hosts (hypovirulence) (Li *et al*., 2016; Mizutani *et al*., 2018).

1.5.4. Resistant plant cultivars

Genetic resistance to FHB is the most efficient and cost - effective approach to attaining substantial, reliable, and sustainable FHB control (Wegulo *et al*., 2015). FHB resistance is a typical quantitative trait, which is regulated by several quantitative trait loci (QTLs), this is why the selection of *Fusarium* resistant plants is very complicated (Rampersad, 2020). In addition, plant resistance to *Fusarium* varies depending on environmental conditions (Nikitin *et al*., 2023). Although there are no fully FHB-resistant cultivars in any cereal species. Five types of FHB physiological resistance can be identified, including (Gagkaeva and Gavrilova, 2011; Timmusk *et al*., 2020): (1) resistance to *Fusarium* propagules penetrating the plant; (2) resistance of the cereal plant to *Fusarium* ear-transmitted disease; (3) resistance of grains against *Fusarium* penetration; (4) overall tolerance of the plant to *Fusarium* infection; and (5) cereal plants potential to accumulate or digest *Fusarium*

mycotoxins. Wheat FHB resistance is not *Fusarium* species-specific; it can be bred for resistance to all *Fusarium* species (Mesterházy *et al*., 2005). So far, up to 500 QTLs distributed over all 21 chromosomes of hexaploid wheat have been identified as QTLs associated with FHB resistance and seven notable QTLs have been well mapped (Fhb1–Fhb7) (Chen *et al*., 2022). There are few cultivars exhibited high level of FHB resistance have been developed in breeding programmes, like sumai 3, frontana, and wangshuibai (Buerstmayr *et al*., 2020). Sumai 3 has a high level of FHB resistance, which is mostly conferred by the Fhb1, Fhb2, and Qfhs.ifa-5A (Fhb5) genes situated on chromosomes 3BS, 6BS, and 5AS, respectively (Chen *et al*., 2022). Moreover, morphological traits like plant height (PH) and anther extrusion (AE) have an impact on FHB infection and can serve as morphological markers to breed FHB-resistant wheat (Chen *et al*., 2022).

1.5.5. Integrated control strategies

There is no individual management strategy will effectively decrease FHB severity or mycotoxin level, although certain management strategies do so, notably if environmental conditions are conducive to disease development (Dweba *et al.*, 2017). Accordingly, integrated disease management strategies are the most effective strategy to manage FHB on cereal crops given the potential for increased reductions in FHB severity and DON contents (Blandino *et al.*, 2012). Integrated management like resistant cultivars, good soil management practices, and local environmental conditions are factors that improve illness control (Scala *et al*., 2016). The use crop rotation, crop rotation + tolerant cultivar, and crop rotation + tolerant cultivar + fungicide application all reduced FHB by 50%, 80%, and 92%, respectively (McMullen *et al*., 2008). A similar study found that the combination of moderately resistant variety, treatment of a triazole fungicide, and ploughing at heading reduced DON on wheat grains contaminated with FHB by 97% (Blandino *et al.*, 2012). In wheat field experiments, the severity of FHB was greatly lessened when *C. flavescens* OH182.9 and *C. aureus* OH71.4 were co-cultured than when either agent was used alone (Schisler *et al.*, 2011). In the effectively integrated management of FHB, BCAs can be co-cultured with other BCAs or used in combination with other control strategies (such as fungicides) (Zhang *et al.*, 2007; Schisler *et al.*, 2011, 2015).

2. Diversity of the *Fusarium* species complexes, the causal agent of FHB disease of durum wheat in Algeria

2.1. Abstract

Fusarium head blight (FHB) occurs in all cereal growing areas of Algeria. Currently, knowledge on the occurrence and phylogenetic diversity of FHB pathogens is lacking. In the present work, eighteen *Fusarium* isolates were isolated from diseased durum wheat seeds and heads from different fields in the north-eastern area of Algeria, and then identified using morphological markers and multi-locus phylogenetic analysis. The isolates were of seven species: *F. clavum*, *F. culmorum*, *F. microconidium*, *F. avenaceum, F. tricinctum, F. solani,* and *F. acuminatum*. The results shows that *F. clavum* was the most common species with 33.3%, followed by *F. acuminatum* (27.7%), *F. culmorum* and *F. avenaceum* with 11.1% each. The least commonly isolated species were *F. microconidium, F. solani,* and *F. tricinctum* with 5.5%. Moreover, sporulation was assessed on PSA, SNA and CLA, showing that CLA was the most efficient medium for spore production of *Fusarium* spp. These data expand our knowledge of species diversity associated with FHB in Algeria and include the first reports of *F. clavum, F. microconidium,* and *F. tricinctum* from durum wheat heads in Algeria, and of *F. microconidium* from durum wheat worldwide.

2.2. Introduction

Cereal crops, notably wheat, barley, maize, and sorghum are primary food supplies in Algeria and other North African countries, where they consumed as flours, raw grains, or manufactured products. The major threats to the worth and production of these economically important crops are cryptogamic diseases like Fusarium head blight (FHB) of wheat. FHB induced by a complex of *Fusarium* species involving at least 19 phytopathogenic species, with *Fusarium graminearum* species complex (FGSC) being the main etiological agent worldwide (Ji *et al*., 2019), which is a subgroup within the *Fusarium sambucinum* species complex (FSAMSC) (O'Donnell *et al*., 2013), including at least 16 phylogenetically different species (Iwase *et al*., 2020), but *F. graminearum* was the most widespread FHB pathogen globally (O'Donnell *et al*., 2008; Sarver *et al*., 2011). Besides the FGSC members, other *Fusarium* species are also related to FHB in the world : *F. culmorum, F. pseudograminearum, F. cerealis* (syn. *F. crookwellense*), and *F. poae* [members of the FSAMSC]; *F. chlamydosporum* [member of the *F. chlamydosporum* species complex (FCSC)]; *F. avenaceum* and *F. tricinctum* [members of the *F. tricinctum* species complex (FTSC)]; *F. equiseti*, [member of the *F. incarnatum–equiseti* species complex (FIESC)] and *F. proliferatum*, *F. subglutinans* and *F. verticillioides* [part of the

Fusarium fujikuroi species complex (FFSC)] (Van Coller *et al*., 2020). Given this context, prevalence and biodiversity of the causal pathogen of FHB of wheat are strongly impacted by the local climate, particularly temperature and humidity.

Algerian climatic conditions, characterized by warm temperatures and relatively high humidity, are conducive to the growth of mycotoxigenic fungal genera, such as *Fusarium, Aspergillus, Alternaria*, and *Penicillium*, in cereal crops and other agri-food commodities. Even though, seldom data are currently recognized about the biodiversity of phytopathogenic and toxigenic Fusaria in Algerian wheat crops, but we could not overlook the risk they can pose to food safety and consumer health.

Fusarium spp. infect plants by the fungus's mycelium or spores, leading to discolouration and shrivelling of grains contaminated with broad spectrum of harmful mycotoxins whether human or animal health, in addition to huge losses in crop yields (Renev *et al*., 2021). Therefore, a significant increase in the frequency and severity of FHB epidemics pose a serious management dilemma to plant pathologists and mycotoxicologists, particularly with the emergence of fungal insensitivity to fungicides applied (Hellin *et al*., 2018; Pasquali *et al*., 2020). Currently, triazoles or benzimidazoles and their combinations are the most effective therapy used to control *Fusarium* spp. diseases (Belabed *et al*., 2022; Francesconi *et al*., 2023).

Morphotaxonomic criteria (macro and micromorphological) serve as the key markers for species-level identification (Leslie and Summerell, 2008). Nevertheless, *Fusarium* taxonomy underwent substantial shifts on the basis of several factors (Crous *et al*., 2021; Wang *et al*., 2022), including phenotypic characters, or phylogenetic inference (O'Donnell *et al*., 2018; Lombard *et al*., 2019a). Furthermore, Fusaria identification to the species level is fraught with many deficiencies: i) morphological overlapping problem due to the significant effect of environmental factors; ii) using single locus datasets (the ITS [internal transcribed spacer] region), which provides a low level of *Fusarium* discrimination between closely related species ; iii) the current *Fusarium* sequences in the NCBI GenBank are primarily misidentified (Aoki *et al*., 2014; Lombard *et al*., 2019b; Wang *et al*., 2019; Xia *et al*., 2019; Crous *et al*., 2021; Yilmaz *et al*., 2021). The diagnosis and management of *Fusarium* diseases have proven to be exceedingly challenging given the above deficiencies, coupled with shifts in the taxonomic framework. Fortunately, the species identification dilemma is circumvented by adopting a polyphasic approach with the combination of morphological markers and multi-gene molecular phylogeny, and by setting up multiple online databases (*Fusarium*-ID, *Fusarium* MLST) (O'Donnell *et al*.,

2015; Crous *et al*., 2021; Torres-Cruz *et al*., 2022). Complex evolutionary relationships of *Fusarium* species, e.g., FIESC, FOSC, and FFSC have been published (Han *et al*., 2023). To date, the genus includes more than 400 phylogenetically distinct species, grouped into 23 clades known as species complexes (O'Donnell *et al*., 2022). Unlike the ITS, a few loci, including translation elongation factor 1-α (*TEF*-1α), have been commonly used as an effective genetic marker for analysing the intra-specific diversity of *Fusarium* spp. owing to its high level of sequence polymorphism between closely related species (Guo *et al*., 2021).

Since such pathogenic fungal species threaten the agricultural system and food security, this work aims to assess the phylogenetic diversity and phenotypic variability of *Fusarium* spp. related to FHB of durum wheat in Algeria.

2.3. Material and methods

2.3.1. Durum wheat sampling and fungal isolation

In 2017/2018, the CNCC (National Center for Seeds and Plants Certification and Control) of Setif state supplied 60 FHB symptomatic durum wheat samples (diseased seeds) of six varieties: Bousselam, Mohamed Ben Bachir (MBB), GTAdur, Cirta, Waha, and Vitron, randomly collected from fields located in various north-eastern provinces of Algeria, including: Batna, Setif, Bordj Bou Arreridj (BBA), M'sila, Khenchela, Biskra and Mila. The samples (each about 250 g) were stored in hermetic paper bags at 4°C for up to 7 days before use to avoid the emergence of more fungal contaminants.

The FHB pathogens were isolated from durum wheat seeds using a method developed by the National Plant Protection Laboratory, France (LSV, 2008). The seeds were superficially disinfected by soaking in 1.5° sodium hypochlorite solution (NaOCl) for 10 min, rinsed three times in sterile distilled water and then dried with sterile filter paper. Seven to eight seeds were deposited onto potato sucrose agar (PSA), and incubated at 25°C ± 3 for 5-7 days. Different types of fungal colonies developed were observed, but only isolates with fusarioid spores were retained for subsequent study.

2.3.2. Purification and conservation of isolates

Pure cultures were obtained from each isolate by successive subculturing on PDA medium. The pure isolates were cultured on potato dextrose agar (PDA) and incubated at 25 ± 3°C for 7 days to perform a monosporic culture as described by Leslie and Summerell, (2008). A spore suspension was serially diluted with sterile distilled water to reach a concentration

of 10^2 spores. mL^{-1}. A drop of this suspension was spread evenly over the Petri dishes containing PDA. After 48 hr incubation at 25 ± 3°C, one germinated spore per isolate was isolated under a stereomicroscope and sub-cultured in a new PDA Petri dish. Plates were incubated at 25 ± 3°C for 7 days.

The two following techniques were used for the conservation of isolates: (i) subculture pure isolates in tubes on PSA slants, incubated at 25 ± 3°C for 7 days, then stored at 4°C, (ii) preservation on filter papers as reported by Singh *et al*. (2018).

2.3.3. Morphological characterization

The morphological characteristics of the fungal pathogen found in FHB durum wheat were defined according to Leslie and Summerell, (2008), Xia *et al*. (2019) and Lombard *et al*. (2019c). All selected isolates were inoculated on both PDA (macroscopic traits) and carnation leaf agar (CLA) (microscopic traits) and incubated at 25°C in darkness for 10 days. Macromorphological characters are mainly appearance and abundance of aerial mycelium, colour colony, pigmentation and sporodochia, whilst micromorphological characters including microconidia (shape, disposition, abundance, conidiophore appearance, branching type of conidiogenous cells), macroconidia (shapes, abundance, basal cell shape, apical cell shape), and chlamydospores were observed in a drop of dye (lactophenol cotton blue) under an optical microscope.

2.3.4. Biometric characterization

The isolates were also characterised by a biometric study based on direct examination under a light microscope to measure the dimensions of a microscopic sample. For this, spore suspension was prepared after 10 days of culture of all isolates on CLA plates to estimate dimensions of spore cells using ocular micrometer, which was calibrated and equipped into one ocular of a microscope at 40x (4x objective). Macroconidia and microconidia mean length and width were calculated from 25 records per isolate.

2.3.5. Sporodochium characterization

For fungus belonging to the genus *Fusarium*, a sporodochium (pI. sporodochia) is similar to small gelatinous clusters on the agar surface, where macroconidia of rather homogeneous shape and size characteristic to the species are formed (LNPV, 2007). For sporodochia characterization, all isolates were grown on CLA, spezieller nahrstoffarmer agar (SNA), and potato sucrose agar (PSA) at 25°C for 10 days to observe the following criteria under a stereomicroscope: disposition, colour, and abundance.

2.3.6. Molecular characterization

To collect mycelia for DNA extraction, isolates were cultured into PDA plates and incubated at 25 ± 3°C for 7 days. mycelia were harvested and the genomic DNA was then extracted using the E.Z.N.A. Fungal DNA Mini Kit (OMEGA, Bio-tek), following the manufacturer's instructions. For molecular identification at the genus level, amplification of the internal transcribed spacers of ribosomal DNA (rDNA-ITS) locus was done using primers pairs ITS1/ITS4 (5'-TCCGTAGGTGAACCTGCGG 3'/5'TCCTCCGCTTATTGATATGC-3') (White *et al*., 1990), while identification at the specie level was done by amplification of the transcription elongation factor 1-alpha (*TEF*-1α) locus using the primers EF1 (5'-ATGGGTAAGGAGGACAAGAC-3') / EF2 (5'-GGAAGTACCAGTGATCATGTT-3') (O'Donnell *et al*., 2004). PCR amplification was performed using the KAPA3G Plant PCR Kit (Kapa Biosystems, Boston, USA). PCR assay was performed in the thermal cycler (T100TM Thermal Cycler; Bio-Rad, Irvine, CA) and PCRs were conducted in 25 µL volume reactions containing 1x buffer, 2.0 mM $MgCl_2$, 0.2 mM each dNTPs, 0.3 µM of each primer, 1U of HOT FIREPol® DNA Polymerase (Solis Biodyne) and 1 µL of fungal suspension from 5 to 7-day-old subcultures in PDA as a template. A non-template negative control was included in each amplification reaction. The thermal cycling parameters for ITS and *TEF*-1α locus were as follows: initial denaturation (95°C, 15 min), denaturation (95°C, 20 sec), annealing (for ITS: 50ºC, 15 sec and for *TEF*-1α: 53ºC, 15 sec), extension (72°C, 1 min) and final extension (72°C, 1 min) for 40 cycles. The PCR products were separated by electrophoresis on 1.5% agarose gels and were visualized by ethidium bromide staining and UV light. Using the MoBio UltraClean® PCR cleanup kit (Carlsbad, CA, USA), positive PCR products were purified according to the manufacturer's instructions, and sent to the BIOfidal laboratory (CEDEX-France) for forward sequencing using sanger dideoxy sequencing (Zimmermann *et al*., 1988). The sequences obtained were compared to those available on the *Fusarium* MLST (https://fusarium.mycobank.org), and NCBI GenBank database (https://www.ncbi.nlm.nih.gov/genbank). The *Fusarium* isolates were identified on the basis of a similarity levels ≥99% among the query and reference sequences.

2.3.7. Phylogenetic analysis

To more effectively resolve the relationships between and within the *Fusarium* species identified, phylogenetic analysis was performed based on the ITS and *TEF-1α* sequences using MEGA 7 software**.** The sequences of *Fusarium* reference strains from GenBank databases using BLAST-N software (http://blast.ncbi.nlm.nih.gov) along with top scoring similarities were selected to build phylograms. The sequences obtained in this study, joined to selected *Fusarium* reference sequences and to sequences of *Fusarium tuaranense* NRRL 46518 and *Fusarium obliquiseptatum* NRRL 62610, used as an outgroup, were aligned using the MUSCLE algorithm (Edgar, 2004) in MEGA 7 software (Kumar *et al*., 2016). Phylogenetic tree was inferred from the combined loci using maximum likelihood (ML) analysis based on the Tamurai-Nei model. Bootstrap (BS) analysis (Felsenstein,1985) with 1000 replications was used to determine the internal branch strength. The phylogenetic tree had a bootstrap values greater than 50%.

2.3.8. Sporulation pattern characterization

To estimate the *in vitro* sporulation capacity of each isolate, three types of culture media were used, namely PSA, SNA, and CLA. A 5-mm-diameter fungal plug from the margin of 1-week-old culture of target isolate grown at 25°C was used to centrally inoculate each replicate and culture medium. The plates were incubated at 25°C for 10 days. The essay consisted of three replicates per medium. Conidial suspensions were prepared by scrubbing gently each colony surface with 10 mL of sterile distilled water containing 0.1% (v/v) tween 20 (for better conidia separation) and then filtering the suspension through two layers of sterile muslin to remove hyphal fragments. Subsequently, 10 µL of suspension is dropped into the "Malassez" cell to count spore density. This procedure is performed three times.

2.3.9. Statistical analysis

In order to further compare the diversity of *Fusarium* isolates included in the study, ANOVA and B Tukey's New Multiple Range Test (P=0.05) were performed for the sporulation pattern and the biometric data of macroconidia and microconidia regarding length and width parameters, by using SPSS 25 software.

2.4. Results

2.4.1. Fungal isolation

The whole endophytic mycobiota isolated from durum wheat grains was comprised of fungal colonies belonging to the following genera: *Alternaria, Aspergillus, Fusarium, Trichoderma, Epicoccum, Penicillium, Rhizopus*, and *Acremonium*. *Fusarium* was the genus with the third highest frequency (18 isolates, 16.66%) of colonies obtained based on colonial and conidial morphology.

The majority of strains were isolated from Mila province (8 strains), followed by BBA and Batna (3 strains), M'sila (2 isolates), and a single strain was recovered from Khenchla and Biskra (Table 2.1, Appendix 4). Although samples from Setif lacked the FHB pathogens, this is not meant to suggest that they couldn't be detected in other locations of this province. The distribution of *Fusarium* strains isolated from each geographical area is shown in Figure 2.1.

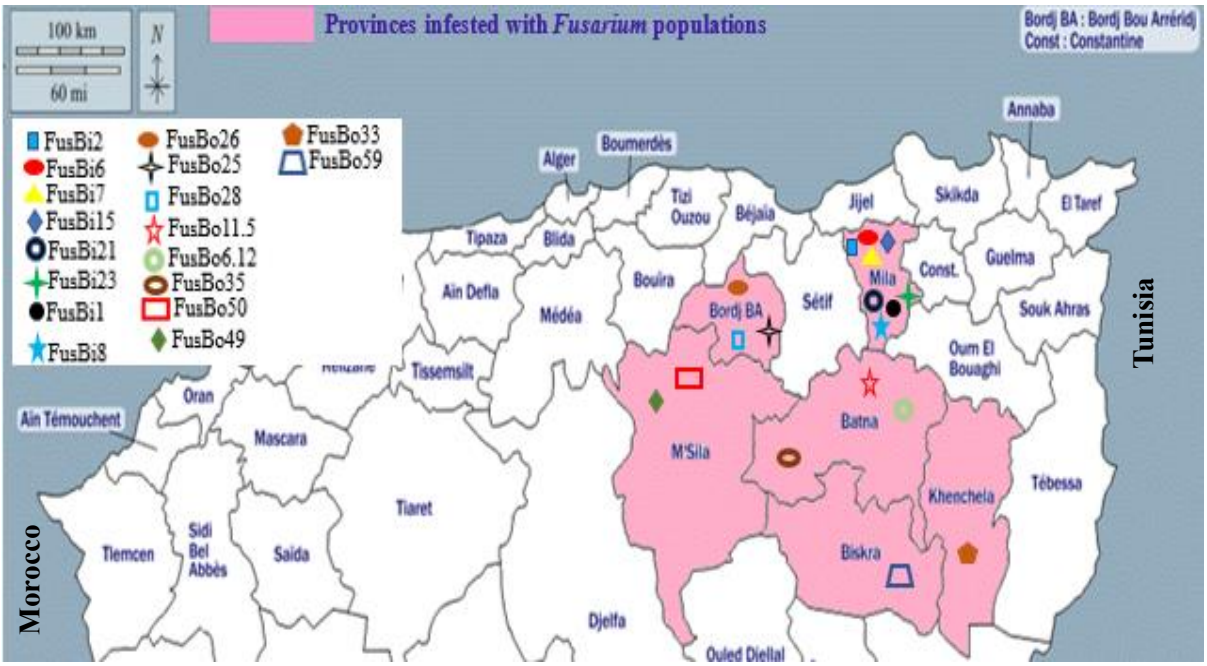


Figure 2.1. Geographical distribution of *Fusarium* strains isolated in the north-eastern region of Algeria.

2.4.2. Morphological characterization

Based on the macro and micromorphological criteria, 18 fungal isolates were identified as belonging to seven *Fusarium* species, *F. clavum* (FusBi8, FusBi1, FusBo25, FusBo28, FusBo49, FusBi2), *F. culmorum* (FusBo50, FusBo59), *F. microconidium* (FusBo26),

Table 2.1. List of *Fusarium* strains isolated from FHB durum wheat samples. The geographical origin, isolate numbers, GenBank accessions, and the identified *Fusarium* species complexes are indicated for each *Fusarium* strain.

Strain code	Strain location	GenBank accessions		*Fusarium* species complex
		ITS	*TEF*-1a	
FusBi8	Bouhatem (Mila)	OR582978	OR569690	FIESC
FusBi1	Bouhatem (Mila)	OR582983	OR569691	FIESC
FusBo25	Sidi M'Barek (BBA)	OR582979	OR569692	FIESC
FusBo28	El Hamadia (BBA)	-	OR569693	FIESC
FusBo50	Ouled Mansour (M'sila)	OR582982	OR569698	FSAMSC
FusBo59	El Haouch (Biskra)	-	OR569697	FSAMSC
FusBo26	Sidi M'Barek (BBA)	OR582984	OR569694	FCSC
FusBi7	Sidi Khelifa (Mila)	OR582977	OR569689	FTSC
FusBi6	Mila (Mila)	OR582976	OR569688	FTSC
FusBi23	Bouhatem (Mila)	OR582980	OR569695	FTSC
FusBo49	Ouled Mansour (M'sila)	-	-	-
FusBo33	Babar (Khenchla)	OR582981	OR569696	FTSC
FusBi2	Redjas (Mila)	-	-	-
FusBi21	Bouhatem (Mila)	-	-	-
FusBi15	Sidi Khelifa (Mila)	-	-	-
FusBo11.5	Tazoult (Batna)	-	-	-
FusBo6.12	Ouyoun El Assafir (Batna)	-	-	-
FusBo35	Tazoult (Batna)	-	-	-

"-" not analysed.

F. avenaceum (FusBi7, FusBi21), *F. tricinctum* (FusBi6), *F. acuminatum* (FusBi23, FusBo33, FusBi15, FusBo11.5, FusBo6.12), and *F. solani* (FusBo35) (Leslie and Summerell, 2008; Xia *et al*., 2019; Lombard *et al*., 2019c). The detailed macromorphological features of the 18 isolates are described in Appendix 2.1. Overall, cultures presented aerial floccose or woolly mycelium, and various pigmentations from pale to dark brown, through red to burgundy were observed. The typical colour of colonies of *Fusarium* isolates grown on PDA are shown in Figure 2.2. Dissimilar macroscopic characters were revealed not only between species, but also between isolates of the same species. All *Fusarium* isolates are illustrated deeply with respect to their microscopic traits

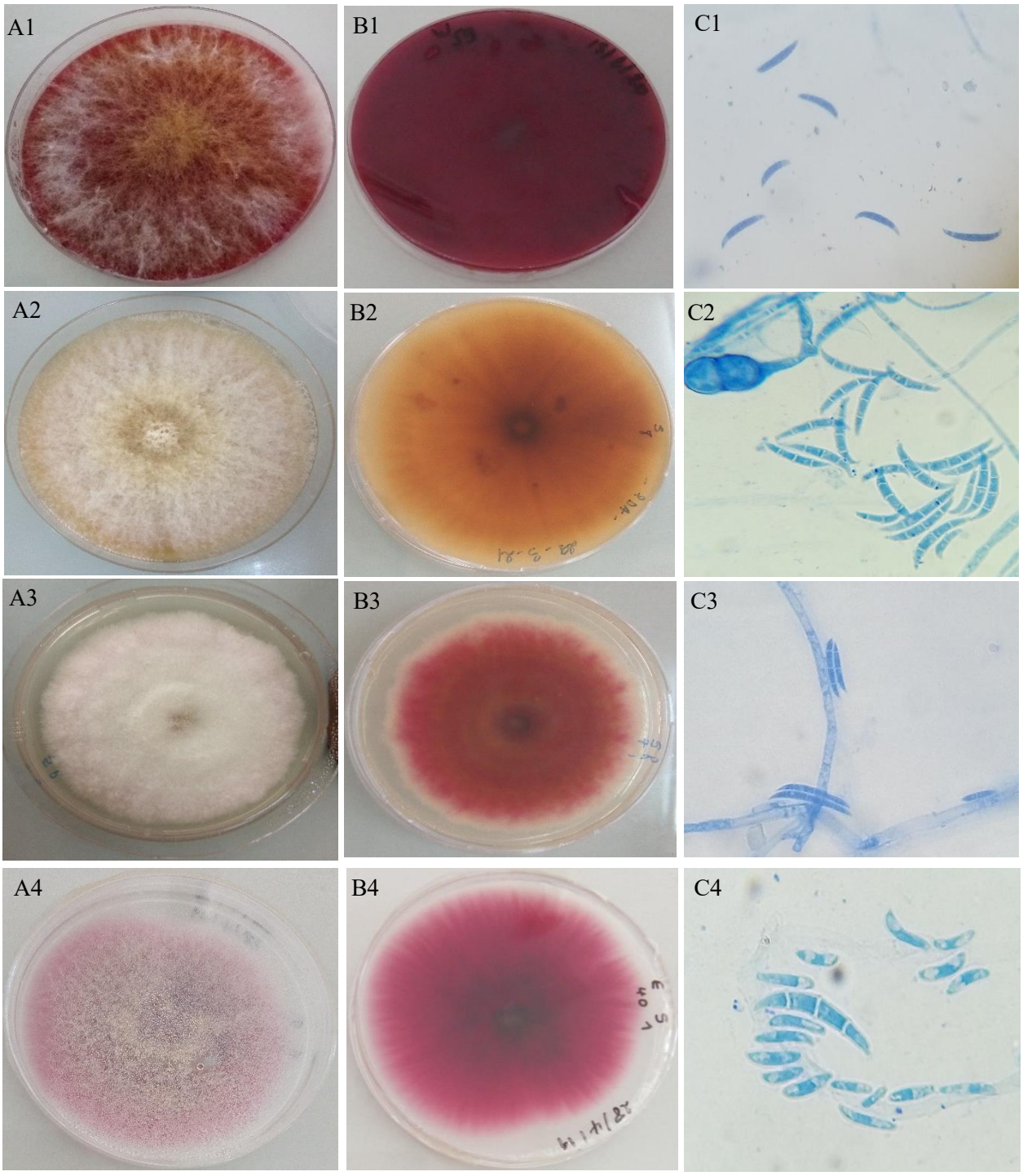


Figure 2.2. Morphological characteristics of morphotype of *Fusarium* species isolated from diseased durum wheat. Each species is depicted by in three pictures (A, B and C). A and B indicated upper and reverse sides of the colony of *Fusarium* strains cultivated on PDA for 7 days, C were micro and macroconidia. Plates 1 to 7 refer to *F. culmorum*, *F. clavum*, *F. avenaceum*, *F. tricinctum*, *F. microconidium*, *F. acuminatum,* and *F. solani* respectively. (40X magnification).

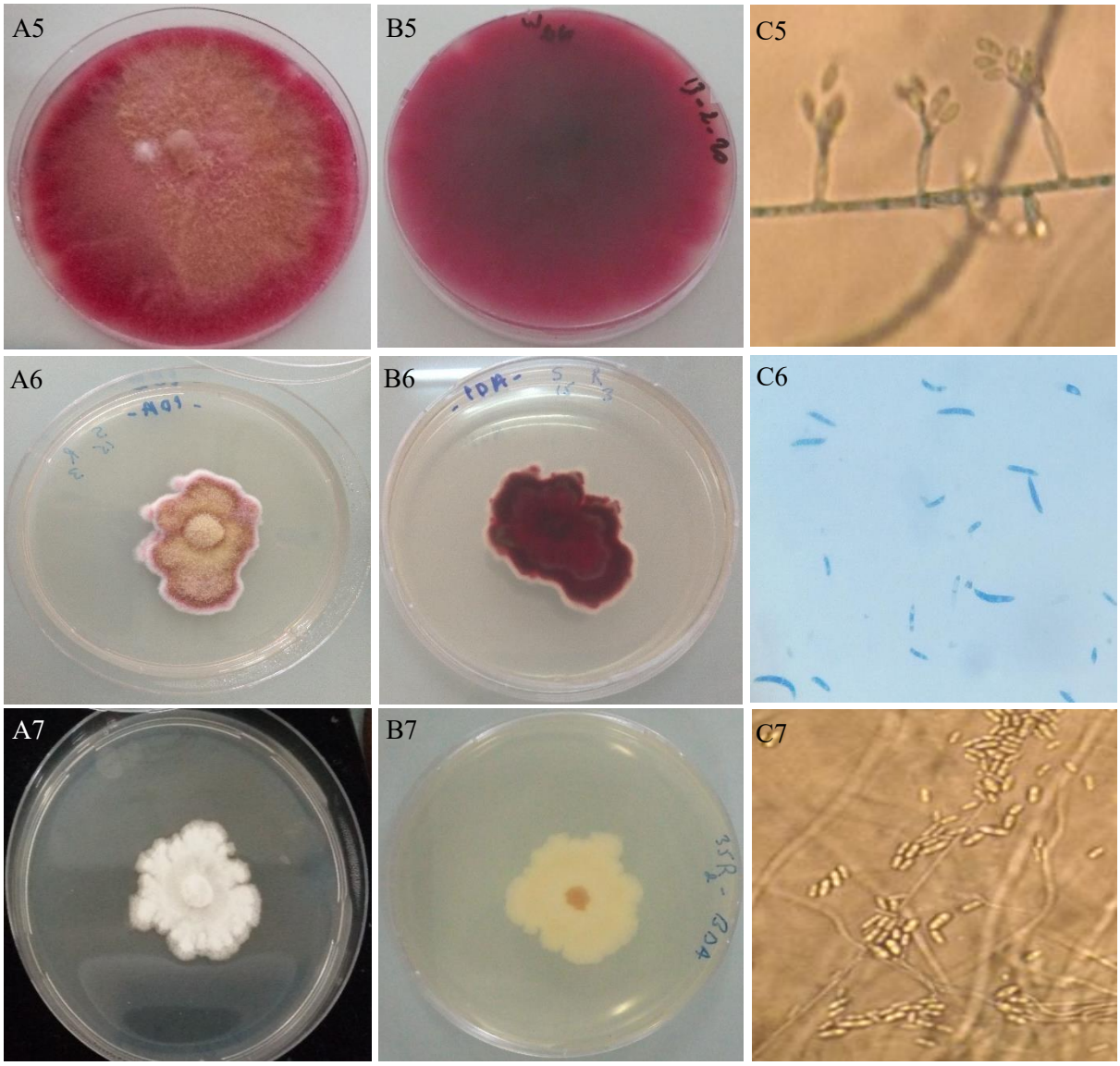


Figure 2.2. (Continued).

(Appendix 2.2, Figure 2.2), which also exhibited a notable contrast among isolates and species.

The species identity was then further confirmed by molecular and multi-gene phylogenetic analyses of a combination of *TEF-1α* and ITS loci.

2.4.3. Biometric characterization

Morphological characteristics of the 18 *Fusarium* isolates on CLA medium revealed a very highly significant difference ($p<0.001$) between macroconidia and microconidia length of the isolates, whereas, no significant difference ($p>0.05$) was detected between macroconidia and microconidia width (Figure 2.2, Table 2.2). The longest macroconidia were produced by the FTSC (*F. avenaceum*, FusBi7) (43.75 ± 3.61 µm) with 3- to 4-

septate, and the shortest (15 ± 0 µm) FIESC (*F. clavum,* FusBo25) with 3-septate. Macroconidia width ranged from 2.5 to 3.75 µm.

The FIESC (*F. clavum,* FusBi8) and FTSC (*F. avenaceum*, FusBi7) isolates showed its characteristic long 1-septate microconidia produced on CLA, 17.5 ± 1.44 and 17.29 ± 3.15 µm respectively, whereas the FCSC (FusBo26) isolate produced the shortest 1-septate microconidia (5 ± 0 µm). Width of microconidia was in the range of 2.5-3.3 µm.

Table 2.2. Macro- and micro-conidia size and septation of 18 *Fusarium* isolates on CLA

	Macroconidia			Microconidia		
Isolates	**Length**	**Width**	**Septum**	**Length**	**Width**	**Septum**
F. clavum						
FusBi8	31.67bcd ± 2.20	3.75a ± 0.72	3-4	17.5d ± 1.44	3.33a ± 0.83	1
FusBi1	21.67abc ± 2.20	2.92a ± 0.42	3	10abc ± 1.44	2.5a ± 0	1
FusBo25	15a ± 0	2.5a ± 0	3	5.83a ± 0.83	2.5a ± 0	1
FusBo28	20ab ± 1.44	2.5a ± 0	3	7.5ab ± 1.44	2.5a ± 0	1
FusBi2	29.17±2.2	2.5±0	3	15.83±2.2	2.5±0	1
FusBo49	15±1.44	3.33±0.83	3	5±1.44	2.5±0	1
F. culmorum						
FusBo50	33.33cd ± 3.63	2.5a ± 0	3-4	/	/	/
FusBo59	31.67 bcd ± 3.00	2.5a ± 0	3-4	/	/	/
F. acuminatum						
FusBi23	21.67abc ± 3.63	2.5a ± 0	5	10abc ± 2.89	2.5a ± 0	1
FusBo33	22.5abc ± 1.44	2.5a ± 0	5	6.25a ± 0.72	2.5a ± 0	0
FusBi15	29.17±3.63	3.33±0.83	5	10±3.82	2.5±0	0-1
FusBo11.5	15±1.44	2.5±0	4	6.25±1.91	2.5±0	0
FusBo6.12	25±1.44	2.5±0	5	6.25±0.72	2.5±0	1
F. microconidium						
FusBo26	/	/	/	5a ± 0	2.5a ± 0	1
F. avenaceum						
FusBi7	43.75d ± 3.61	3.75a ± 0	3-4	17.29d ± 3.15	2.92a ± 0.42	1
FusBi21	40.83±3.63	2.92±0.42	4-5	15±1.44	2.5±0	1
F. tricinctum						
FusBi6	15.83a ± 2.20	2.5a ± 0	3	7.5ab ± 1.44	2.5a ± 0	0
F. solani						
FusBo35	25±1.44	2.5±0	5	9.17±0.83	5±0	0

Different lowercase letters in the same column indicate significant differences analysed by ANOVA followed by Tukey's HSD test ($p \leq 0.05$). Data are mean ± SEM (n = 25).

/= No conidia.

2.4.4. Sporodochium characterization

The effect of several culture media, including PSA, SNA, and CLA on the sporodochia pattern of *Fusarium* isolates was studied. Isolates showed variable sporodochia production on different media, but the optimum production medium was SNA over others. The lowest

production was noted on CLA medium. Generally, sporodochia were characterised by diverse colours comprising pale rose, white, transparent, orange, pale orange, beige, yellow, or cream (Appendix 2.3, Figure 2.3).

2.4.5. Molecular characterization and phylogenetic analysis

PCR using primers pairs of ITS1/ITS4 and EF1/EF2 was successful with 11 isolates, and yielded in bands of 500 bp (Figure 2.4b) and 700 bp (Figure 2.4a), respectively. All amplification by the *TEF*-1α was successful. Nevertheless, a total of seven isolates were not successfully sequenced, but they were morphologically identified. These included three isolates (FusBi15, FusBo11.5, FusBo6.12) of *F. acuminatum*, two isolates (FusBo49, FusBi2) of *F. clavum*, one isolate (FusBi21) of *F. avenaceum*, and one isolate (FusBo35) of *F. solani*. Based on GenBank and Fusarium MLST databases, 11 isolates were identified as *F. acuminatum* (FusBi23, FusBo33), *F. incarnatum-equiseti* species complex (FIESC) (FusBi8, FusBi1, FusBo25, FusBo28), *F. avenaceum* (FusBi7), *F. culmorum* (FusBo50, FusBo59*), F. chlamydosporum* species complex (FCSC) (FusBo26), and *F. tricinctum* species complex (FTSC) (FusBi6). DNA sequences for 11 *Fusarium* isolates have been deposited in GenBank under the accession number listed in Table 2.1. Our maximum likelihood bootstrap analysis (Figure 2.5) revealed the phylogenetic position of pathogenic isolates, which clustered in four well-supported clades, corresponding to the *F. incarnatum-equiseti* species complex (FIESC), *F. sambucinum* species complex (FSAMSC), *F. chlamydosporum* species complex (FCSC), and *F. tricinctum* species complex (FTSC). The FCSC was represented by only one strain (FusBo26) identified as *F. microconidium*. FTSC clade included one strain (FusBi7) that had high similarity to the *F. avenaceum* reference strain, one strain (FusBi6) to *F. tricinctum*, and two strains (FusBi23, FusBo33) to *F. acuminatum*. Four FIESC strains (FusBi8, FusBi1, FusBo25, FusBo28) clustered with *F. clavum* CBS 126202. Another two strains (FusBo50, FusBo59) belonged to the FSAMSC, recognised as *F. culmorum*.

Eighteen *Fusarium* isolates were identified in this study, divided among seven *Fusarium* species. The prevalence of the different *Fusarium* species complexes determined by phylogenetic analysis of the combined data set of ITS and *TEF*-1α loci in FHB durum wheat is indicated in Figure 2.6. The FTSC and FIESC from the present study were most prevalent (47% and 35% of isolates, respectively), followed by the FSAMSC, with 12%. Only a single (6%) isolate belonging to the FCSC was detected.

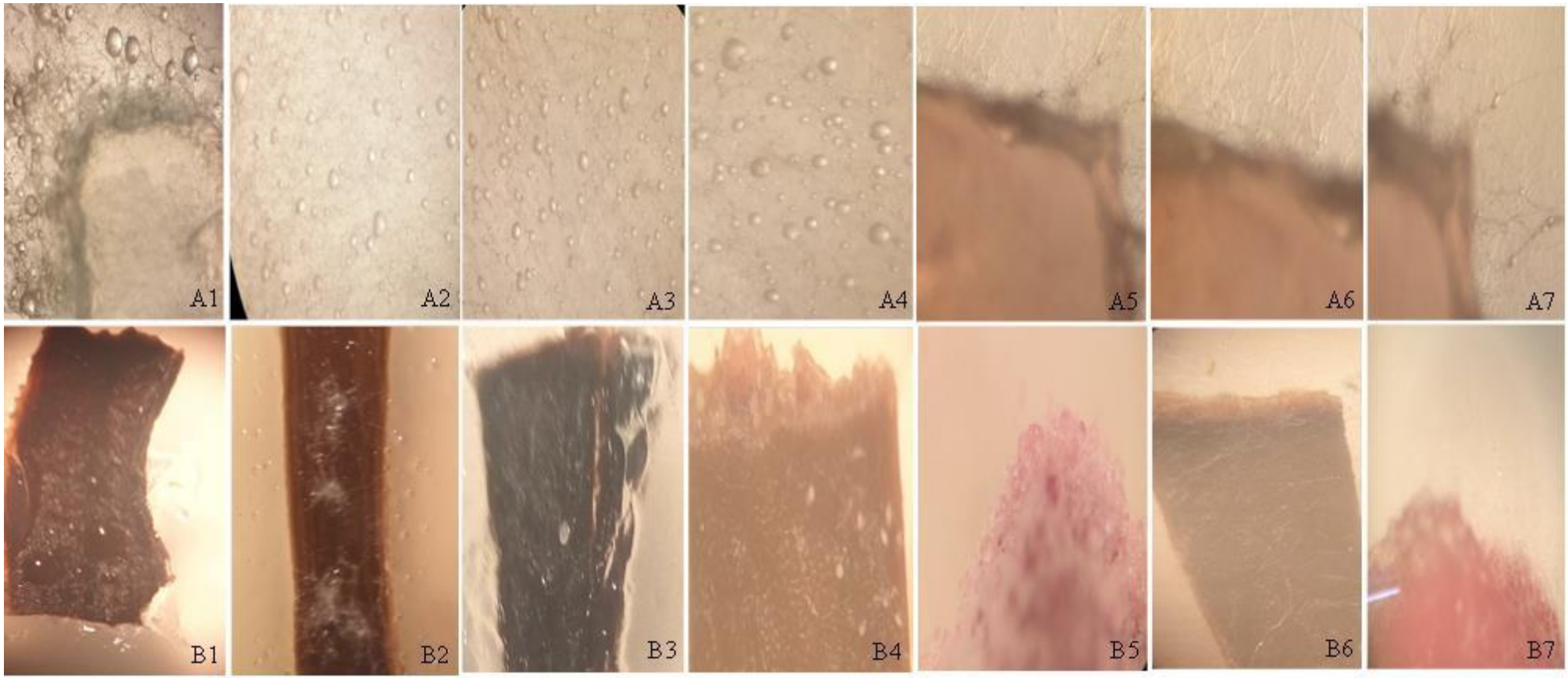


Figure 2.3. Appearance of sporodochia produced by *Fusarium* species on SNA and CLA media. Each species is depicted by in two pictures (A and B). A and B indicated the sporodochia produced on SNA and CLA media for 7 days. Plates 1 to 7 refer to *F.clavum*, *F. acuminatum*, *F. avenaceum*, *F. culmorum*, *F. tricinctum*, *F.microconidium*, and *F. solani,* respectively.

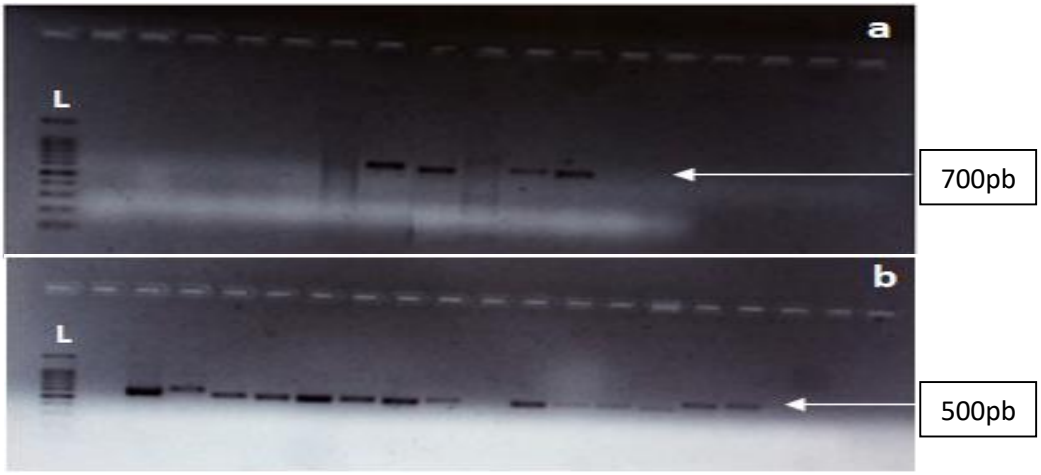


Figure 2.4. Electrophoresis product of amplified DNA of isolates of *Fusarium* spp. **a.** Amplification with universal primers EF1/EF2, **b.** Amplification with universal primers ITS1/ ITS4. **L:** Ladder.

2.4.6. Sporulation pattern characterization

The quantity of conidia produced by the 18 isolates yielded a very highly significant difference (p<0.001) between the three nutrient media examined after 10 days of incubation (Table 2.3). For all isolates, CLA produced conidia with peak production on day 10 (1.49×10^7 conidia. mL^{-1}), whilst the lowest production was recorded on SNA medium, with average level of around 3.05×10^5 microconidia. mL^{-1}. Sporulation on PSA was lower than that on CLA medium, with mean content of conidia being 6.5×10^5 conidia. mL^{-1} (Figure 2.7).

The *F. acuminatum* isolate (FusBo11.5) sporulated around $26.67 \pm 0.001 \times 10^7$ conidia. mL^{-1} on CLA. Nonetheless, a number of isolates reacted distinctly to the medium, by not or rarely forming conidia, proving that the impact of the medium on conidiation was isolate dependent. An interesting observation was the poorest sporulation (from 0 ± 0 to $0.015 \pm 0.002 \times 10^5$ conidia. mL^{-1}), which singularised *F. acuminatum* isolate (FusBo33) from the others, whatever the medium used, likely ascribed to its intrinsic character (Table 2.3).

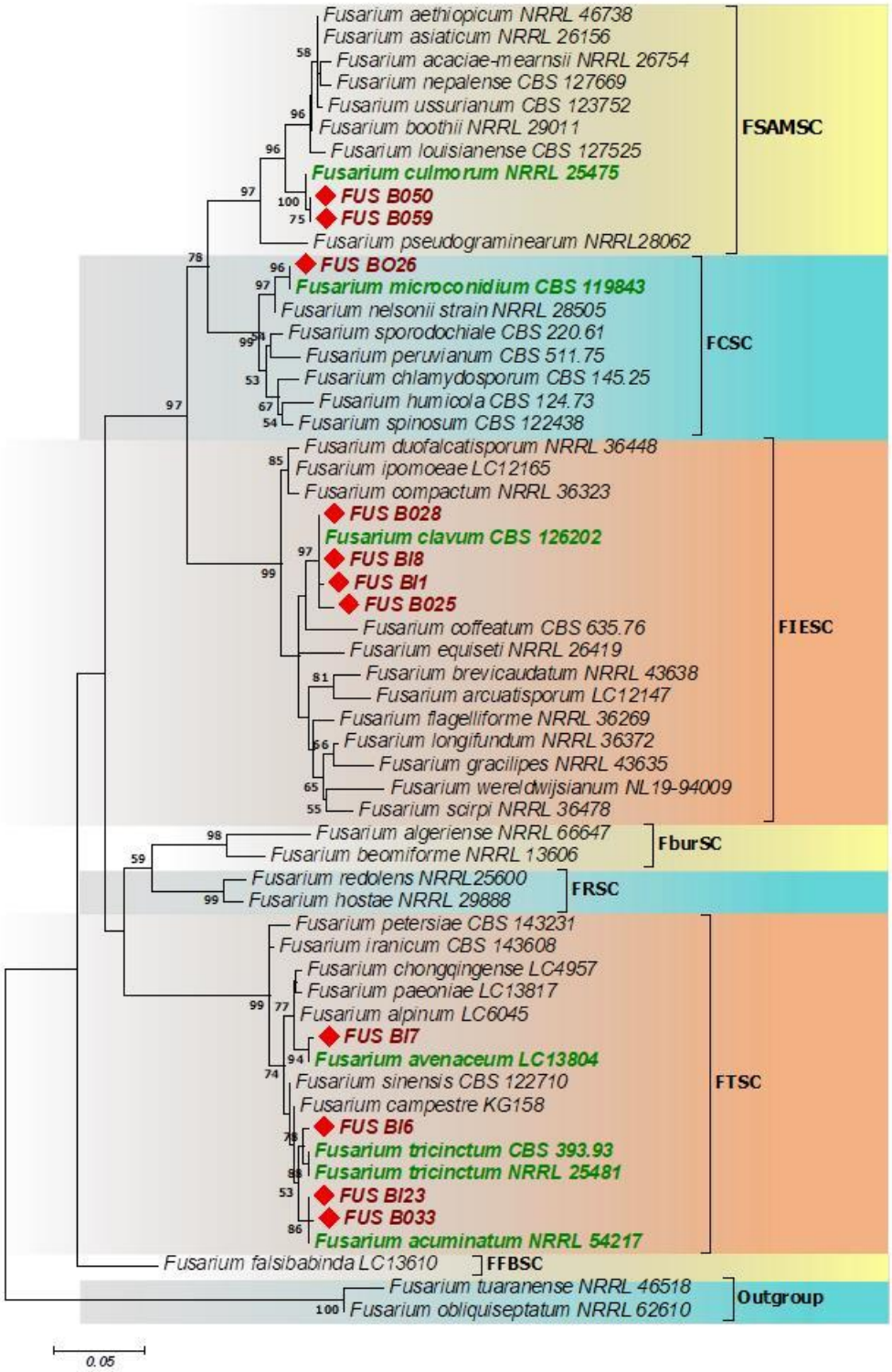


Figure 2.5. Maximum-likelihood phylogenetic tree inferred from the combined data set of ITS and *TEF*-1α loci of 11 *Fusarium* isolates. Bootstrap values greater than 50% are indicated on the nodes. The phylogram is rooted with *Fusarium tuaranense* NRRL 46518 and *Fusarium obliquiseptatum* NRRL 62610. *Fusarium* strains included in this study are in red. *Fusarium* species complexes are presented in the clustering.

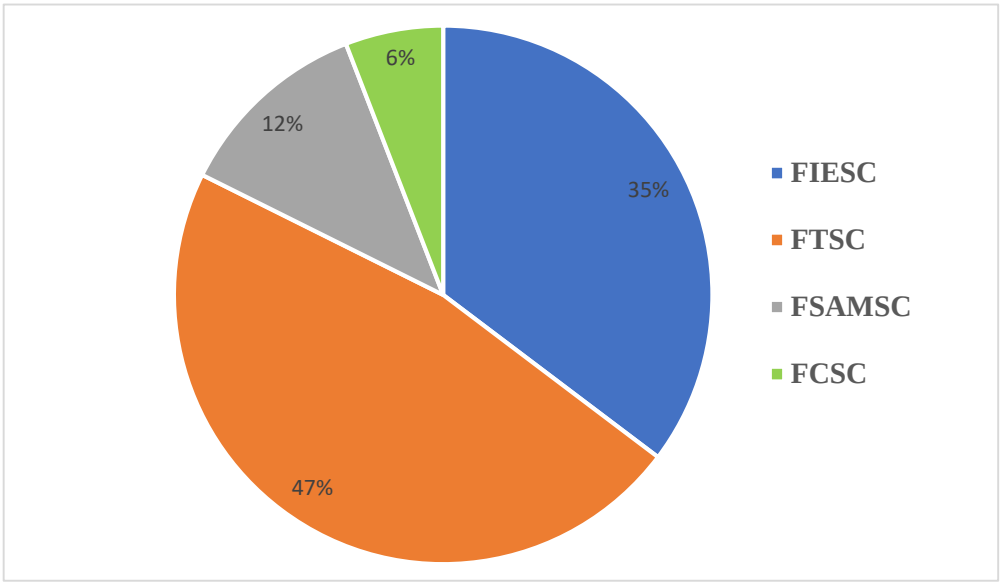


Figure 2.6. Prevalence of *Fusarium* species complexes, phylogenetically identified by combined data set of ITS and *TEF*-1α loci, in Algerian FHB durum wheat.

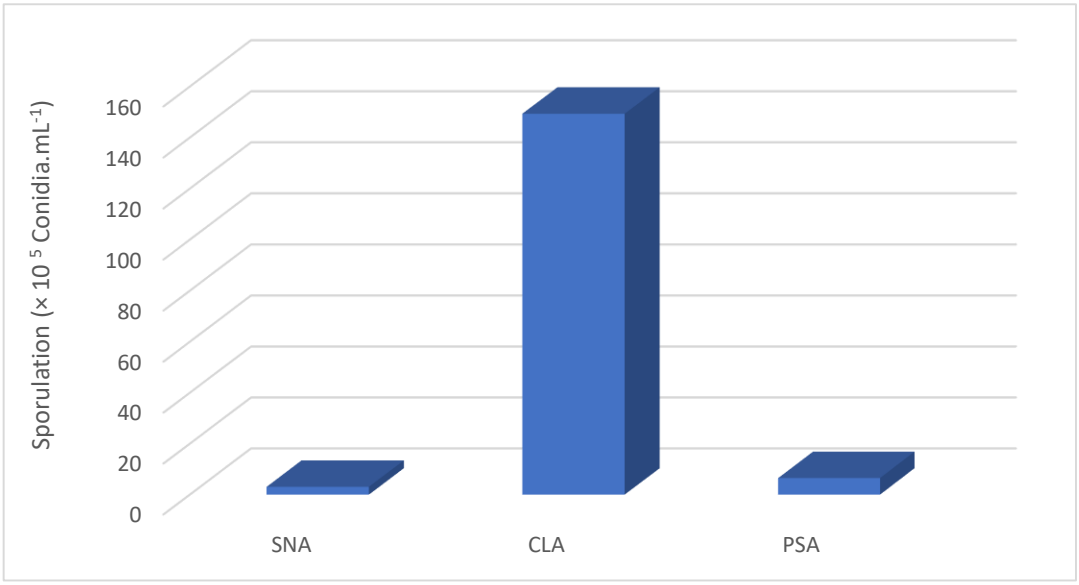


Figure 2.7. Sporulation rates of *Fusarium* isolates in different culture media.

Table 2.3. Variability in the sporulation rates of *Fusarium* isolates in different culture media.

Isolates	Sporulation (10^5 Conidia. mL^{-1})		
	SNA	**CLA**	**PSA**
F. clavum			
FusBi8	2.266±0.290a	12.066±4.043b	13.6±0.115bc
FusBi1	0.058±0.004a	0.021±0.000a	0.496±0.008a
FusBo25	2.866±0.520a	0.033±0.003a	21±0.577c
FusBo28	1.333±0.176a	0.13±0.011a	0.186±0.033a
FusBi2	0.416 ±0.008	0.007 ±0.001	0.233 ±0.008
FusBo49	1.666 ±0.352	0 ±0	2.333 ±0.066
F. culmorum			
FusBo50	2.133±0.176a	2.4±0.642a	0.04±0.005a
FusBo59	1.133±0.066a	1.4±0.305a	2.2±0.305a
F. acuminatum			
FusBi23	0.02±0.01a	0.056±0.016a	0.036±0.003a
FusBo33	0.015±0.002a	0 ± 0a	0.009±0.000a
FusBi15	0.016 ±0.003	0.266 ±0.054	0.036 ±0.003
FusBo11.5	0.633 ±0.088	2666.666 ±0.176	0.016 ±0.003
FusBo6.12	0.633 ±0.104	0.060 ±0.010	3.466 ±0.466
F. microconidium			
FusBo26	39±7.371b	3.933±0.819a	64±8.082d
F. avenaceum			
FusBi7	2.6±0.230a	0.153±0.021a	1.233±0.033a
FusBi21	0.110 ±0.026	0.036 ±0.017	0.002 ±0.001
F. tricinctum			
FusBi6	0.043±0.006a	2.266±0.133a	9.366±0.120ab
F. solani			
FusBo35	0.030 ±0.010	0.406 ±0.049	0.030 ±0.005

Different lowercase letters in the same column indicate significant differences analysed by ANOVA followed by Tukey's HSD test ($p \leq 0.05$). Data are mean ± SEM (n = 3).

2.5. Discussion

This is the first study to adopt a polyphasic approach combining morphological and multi-locus phylogenetic identification to resolve the phylogeny of *Fusarium* isolates recovered from FHB of durum wheat in Algeria. Eighteen *Fusarium* isolates were detected and identified to seven species (*F. clavum*, *F. culmorum*, *F. microconidium*, *F. avenaceum, F. tricinctum, F. solani,* and *F. acuminatum*). According to analyses based on Genealogical Concordance Phylogenetic Species Recognition (GCPSR; [Taylor *et al*., 2000]), the *Fusarium* genus consists of 23 species complexes (O'Donnell *et al*., 2015; Summerell, 2019). Four distinct species complexes occurred among our isolates, FCSC represented by *F. microconidium*, FTSC included *F. avenaceum, F. tricinctum,* and *F. acuminatum*, FIESC represented by *F. clavum,* and FSAMSC included *F. culmorum*, pointing to that a significant *Fusarium* biodiversity is present in Algeria. This is the first report that *F. clavum, F. microconidium,* and *F. tricinctum* are associated with FHB of durum wheat.

Obvious differences in colony morphology, notably colony colour, were observed among the isolates (Figure 2.2, Appendix 2.1). In addition, there was a significant difference ($p<0.001$) between macroconidia and microconidia size (Figure 2.2, Table 2.2). The size and septation of macro- and microconidia were in line with previous findings on the morphological description of *Fusarium* spp. (Leslie and Summerell, 2008; Xia *et al*., 2019; Lombard *et al*., 2019c). The mycelia of *Fusarium* isolates were floccose or woolly, extremely abundant, abundant, or moderate, with pigmentations ranging from pale to dark brown, through red to burgundy. The various colours of colonies of *Fusarium* spp. on PDA were depicted in Figure 2.2. Indeed, sporodochia were distinguished by a wide range of colours, like pale rose, white, transparent, orange, pale orange, beige, yellow, or cream (Figure 2.3). Moreover, the isolates produced sporodochia variably on several culture media (CLA, SNA, and PSA), although the best media were PSA and SNA, which was in agreement with previous findings from *Fusarium* diversity and phylogenomic studies (Ujat *et al*., 2021; Hassan *et al*., 2023).

For classification of *Fusarium* species, preliminary identification is mainly performed against conventional methods. However, such methods could neither reveal intraspecific diversity nor distinctly discriminate among closely related species. Therefore, the use of phylogenetic analyses based on molecular characteristics is required to efficiently identify similar strains of the causal agent, leading to more grasp their population structure and variability.

As reported here, a combination of the ITS and *TEF*-1α loci was phylogenetically informative in identifying our isolates into the previously described species and species complexes. In north-eastern provinces, *F. clavum,* a FIESC 5 phylospecies was the most common species with 33.3% frequency, followed by *F. acuminatum* (27.7%), *F. culmorum* and *F. avenaceum* with a frequency of 11.1% each. The least commonly isolated species were *F. microconidium, F. solani and F. tricinctum* with 5.5% frequency. The results indicate that *F. clavum* from the FIESC was the most common. As was the case with a study of FIESC isolates from Spanish wheat (Castellá and Cabañes, 2014) and from Italian wheat (Villani *et al*., 2016). However, *F. culmorum* and *F.pseudograminearum* are the two prevalent species in further northern Algerian provinces (Abdallah-Nekache *et al*., 2019; Hadjout *et al*., 2022). Although *F. culmorum*, *F. graminearum,* and *F. poae* are the dominant contributor to FHB in Europe (Senatore *et al*., 2021). Each *Fusarium* species exhibits a distinct level of environmental tolerance (Abdel-Azeem *et al*., 2019). For example, *F. acuminatum* and *F. culmorum* usually occur in regions with moderate climate and cannot thrive at temperatures beyond +25°C (Gagkaeva *et al*., 2014). *F. avenaceum* was typically recovered in cooler climates (Stakheev *et al*., 2016).

FIESC exhibit high genetic diversity, currently comprising 38 recognised phylospecies (FIESC 1-38) from a wide variety of habitats/hosts worldwide (O'Donnell *et al*., 2009, 2018; Short *et al*., 2011; Villani *et al*., 2016, 2019; Avila *et al*., 2019; Hartman *et al*., 2019; Maryani *et al*., 2019; Santos *et al*., 2019; Wang *et al*., 2019; Xia *et al*., 2019; Lima *et al*., 2021). In this study, six isolates identified as *F. clavum* (FIESC 5). The *TEF*-1α has been widely used to resolve phylogenetic relationships within FIESC, as it is a highly informative marker locus (O'Donnell *et al*., 2009; Santos *et al*., 2019). The morphological description of *F. clavum* in this study belonging to FIESC is in agreement with that given by Xia *et al*. (2019), and Manganiello *et al*. (2021). These findings provide vital background data to more effectively predict the potential threat of *F. clavum* as a toxigenic and pathogenic species and to develop preventive strategies intended to mitigate high yield losses in Algerian fields. As matter of fact, FIESC 5 strains have been reported to produce toxins, including DON, DON derivatives, DAS, NIV, NEO, and FUS-X, though type A thricothecenes T-2 and HT-2 were not produced (Marin *et al*., 2015).

F. clavum was described as a new taxon by Xia *et al*. (2019). As far as we are aware, this is the first report of *F. clavum* associated with FHB of durum wheat in Algeria.

FTSC includes at least 15 phylospecies (Senatore *et al*., 2021). In this study, eight isolates comprising three FTSC phylospecies, which are listed here in decreasing incidence: *F.*

acuminatum (FTSC 2, N =5), *F. avenaceum* (FTSC 4, N = 2), and *F. tricinctum* (FTSC 3, N = 1). The recovery of these species reveals a bothersome issue owing to their ability to produce significant levels of multiple "emerging" mycotoxins, mainly moniliformin (MON) and enniatins (ENNs), 2- amino-14,16-dimethyloctadecan-3-ol (AOD-ol) and other secondary metabolites including chlamydosporol (CHL), acuminatopyrone (ACU), longiborneol (LONG), fungerin (FUNG), and BUT (butanolide) (Senatore *et al*., 2021),and they also produce DON (Belabed *et al*., 2023), which could contaminate Algerian cereals and deteriorate wheat quality.

FSAMSC comprises members of the FGSC, which are associated with FHB epidemics on agriculturally important crops, especially small grain cereals. Two isolates in this study belonged to the FSAMSC, identified as *F. culmorum*, well known as a pathogen of diverse host plants, mainly cereals, like wheat, barley, sorghum corn, and oats. Moreover, the toxin profile reported for this species indicate production of zearalenone (ZEA), deoxynivalenol (DON) and its derivatives, nivalenol (NIV), ENNs, MON, culmorin (CUL) and hydroxy culmorin (OHCUL) (Beccari *et al*., 2018; Laraba *et al*., 2021; Belabed *et al*., 2023). These mycotoxins can lead to immunosuppression, nephrotoxicity, hepatotoxicity, gastrointestinal complaints, and other health concerns.

FCSC now has nine phylospecies (Lombard *et al*., 2019c). Only one FCSC strain in this study clustered with *F. microconidium*, which was described as a new taxon of unknown origin and substrate, with culture ex-type (CBS 119843 = MRC 8391=KSU 11396) (Lombard *et al*., 2019c). More recently, *F. microconidium* was recorded inducing leaf spot disease on date palms in Oman (Al-Nabhani *et al*., 2023). To the best of our knowledge, this is the first report of *F. microconidium* associated with FHB of durum wheat in Algeria, and of *F. microconidium* from durum wheat worldwide. Follow-up studies are therefore warranted to further assess the pathogenicity of this novel phylospecies on durum wheat and other small grain cereals, and to ascertain whether this species can produce mycotoxins.

Strain competitiveness is defined by the ability to produce spores, which serves both as the main vehicle for spreading fungal diseases and as a form of resistance under harsh conditions, such as nutrient starvation. For *Fusarium* isolates tested, CLA medium supported the highest sporulation, which will be useful for further *Fusarium* taxonomy, mycotoxins and pathogenicity studies. CLA is among the low-nutrient media that have been proven to be the most suitable for the induction of sporulation in *Fusarium* (Leslie and Summerell, 2008), as indicated by Lazarotto *et al*. (2014) who showed higher spore

production of *Fusarium chlamydosporum* species complex isolates in CLA, especially under continuous light. In contrast, these findings differ from others given by Zhao *et al*. (2023), where potato sucrose liquid medium (carbohydrate-rich medium) resulted in a higher sporulation rate of *F. equiseti*. The sporulation process in fungi is controlled by a complex system of positive and negative gene regulators, which are influenced by environmental and nutritional factors (Ajdari *et al*., 2011).

Discrepancies in conidia content were revealed not only between species, but also between isolates of the same species on different nutrient media as seen in the present study, which is interpreted by the genetic differences in the species/isolates to use the nutrients supplied. In this study, CLA proved to be the most effective medium for sporulation of *Fusarium* spp. recovered from FHB of durum wheat. The significance of this study is that it sheds light on the diversity of *Fusarium* species and species complexes associated with FHB of durum wheat, which lays the groundwork for future research and could help to implement effective control strategies against threats posed by toxigenic and pathogenic Fusaria in order to safeguard consumer health.

2.6. Conclusion

Based on polyphasic analysis, the collected strains from FHB of durum wheat in Algeria were identified to seven species as *F. clavum*, *F. culmorum*, *F. microconidium*, *F. avenaceum, F. tricinctum, F. solani,* and *F. acuminatum*. The analysis offered the first evidence for the occurrence of four *Fusarium* species complexes, with the FIESC and FTSC as the most common. This study emphasizes the importance of an accurate identification of pathogen species, which pose a growing threat to the global agricultural system and food security. To our knowledge, this is the first report that *F. clavum, F. microconidium,* and *F. tricinctum* are associated with FHB of durum wheat.

3. Pathogenicity and mycotoxin profile of *Fusarium spp.* inducing wheat head blight in Algeria

3.1. Abstract

Fusarium is one of the world's most harmful wheat pathogens, causing severe crop diseases such as Fusarium head blight (FHB). These fungi pose a significant threat to wheat production by considerably reducing crop yield, quality, and safety through grain contamination with mycotoxin. The focus of the current study emphasizes aspects related to the pathogenicity and toxigenicity of some FHB isolates recovered from symptomatic Algerian wheat samples. Three durum wheat genotypes were subjected to two pathogenicity tests (*in vitro* and *in vivo*), and the results showed significant variations in aggressiveness across various phenotypic parameters, suggesting intrinsic genetic variation in the host-pathogen interaction. *In vivo,* all *Fusarium* isolates were pathogenic fulfilling Koch's postulates. The *Fusarium* isolates were tested for their mycotoxinogenicity and were found to produce deoxynivalenol, zearalenone, and T-2 toxin. Among these isolates, *F. culmorum* FusBo59 exhibited the highest toxicity, produced DON as the prevalent mycotoxin, with maximum level equal to 7.128 $\mu g.kg^{-1}$ in the ELISA assay and a staggering 373196.19 $\mu g.kg^{-1}$ by LC-MS/MS, surpassing the European threshold of 1750 $\mu g.kg^{-1}$. Toxin profiling revealed that 15-ADON (63.6%) predominated in wheat grains, followed by DON (18.2%) and 3-ADON (9.1%). T-2 and zearalenone were present but remained below the EU limits of 100 $\mu g.kg^{-1}$. Moreover, *Aspergillus* mycotoxin namely AFB2 occurred with mean concentration of 18.5 $\mu g.kg^{-1}$which is over the EC limit (4 $\mu g.kg^{-1}$). In contrast, *F. avenaceum* FusBi7 emerged as the most aggressive isolate, while the Cirta variety exhibited the highest tolerance to *Fusarium* attacks. These findings underscore the independent evolution of disease induction and toxin production among *Fusarium* isolates.

3.2. Introduction

Wheat, a staple food consumed worldwide, is a significant source of energy and nutrition, providing 20% of global calories and protein (FAO, 2018). Unfortunately, wheat is often subject to many pathogens under favorable environmental conditions throughout its production cycle, from harvesting to transport and storage.

Fusarium spp. are the most harmful wheat pathogens worldwide, inducing severe crop diseases such as Fusarium head blight (FHB), Fusarium root rot (FRR), and Fusarium crown rot (FCR), all of which have negative consequences for food safety and security, agricultural production, and human and animal health. FHB can cause significant economic losses in wheat by causing grains to whiten early, wilt, discolor, and the demise of spikelets or complete heads (Petronaitis *et al.,* 2021). Moreover, *Fusarium* spp. contaminate cereals with multiple mycotoxins such as trichothecenes, deoxynivalenol (DON), nivalenol (NIV), T-2 toxin (T-2), HT-2 toxin (HT-2),

diacetoxyscirpenol (DAS), and zearalenone (ZEA), which are known to be a threat to global public health through dietary exposure and also have a significant effect on pathogen fitness and food safety (Adnan *et al*., 2020; Wang *et al*., 2020). In this monocyclic disease, the level of aggressiveness is frequently measured by assessing disease severity. Higher levels of aggressiveness are associated with earlier symptom evolution and higher levels of mycelium and mycotoxins in plant tissue (Miedaner *et al*., 2004). In this context, the ability of *Fusarium* mycotoxins to be produced varies not only between species but also between strains of the same species. The level of mycotoxin accumulation in infected wheat grain, however, can vary due to climatic factors, cultivation system, method, and date of grain harvesting, as well as the degree of FHB-resistant wheat cultivars (Golinski *et al*., 2010; Bernhoft *et al*., 2012; Xu *et al*., 2014; Del Ponte *et al*., 2015). According to the Food and Agriculture Organization (FAO), approximately 25% of the world's food crops are contaminated by mycotoxins, with detectable mycotoxins expected to reach 60-80% in all food and feed crops globally by 2020 (Eskola *et al*., 2020). Currently, up to 500 different forms of mycotoxins have been found (Haque *et al*., 2020).

The most important mycotoxins in terms of food safety and legislation include aflatoxins (AFs), ochratoxin A (OTA), DON, fumonisins (FUMS), ZEA, ergot alkaloids, T-2, HT-2, patulin, and citrinin (Eskola *et al.*, 2020). Human exposure to mycotoxins is acquired directly by ingesting contaminated plant-origin products such as cereals, dried fruits, and nuts or indirectly by eating animal-origin products such as contaminated milk, eggs, and meat (Capriotti *et al*., 2012; Flores-Flores *et al.*, 2015), leading to several dramatic health hazards, including cancer, immunosuppression, nephrotoxic, hepatotoxic, mutagenic, estrogenic, and gastrointestinal effects. FHB pathogen species can be classified based on the profile of toxic secondary metabolites produced, which can result in diverse chemotype profiles that can cause different forms of grain infection depending on the occurrence of each species in the crop.

According to several studies, chemotypes that produce DON, 3-acetyldeoxynivalenol (3-ADON), and 15-acetyldeoxynivalenol (15-ADON) are more virulent in plants than those that produce NIV (Gilbert *et al*., 2010; Puri and Zhong, 2010; Pasquali *et al*., 2016). Trichothecene compounds (TCT) (particularly DON), ZEA, and FUMS are the most typically produced by *Fusarium* spp. in wheat (Lemus-Minor *et al.*, 2015; Sadhasivam *et al.*, 2017). *Fusarium graminearum*, for example, is the primary producer of type B trichothecenes (Type B TCT) (primarily DON and its acetylated forms 3-ADON and 15-ADON), whereas *F. sporotrichioides* and *F. langsethiae* are the primary producers of type A trichothecenes (T-2 and HT-2) (McCormick *et al*., 2011). Furthermore, the new A-trichothecene NX-2 is produced by a small

percentage of *F. graminearum* isolates from the United States and Canada (Liang *et al*., 2014; Kelly *et al*., 2016; Kelly and Ward, 2018).

Given this diversity, crop protection against *Fusarium* spp. and associated mycotoxins is critical for ensuring the quality and safety of grains meant for consumption as seed, feed, and edibles and refining management techniques to protect consumer health. There was a correlation between DON accumulation and the level of FHB symptoms in one trial, where DON-resistant cultivars also demonstrated *Fusarium* resistance (Lemmens *et al*., 2016); however, in another, near to negative associations were detected (Ji *et al*., 2015). Furthermore, there was no link between grain's level of mycotoxin contamination and the visual evaluation of etiological symptoms (Birzele *et al*., 2002).

As a fundamental immunoassay, the screening ELISA kit is the conventional analytical tool for mycotoxin determination due to its simplicity, specificity, and cost-effectiveness. However, advanced analytical approaches have been developed for the more precise and sensitive analysis of mycotoxins. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the preferred method due to its high selectivity, sensitivity, and rapid multi-mycotoxin analysis. Despite its prominence as a staple crop, research into the pathogenicity and mycotoxin levels of *Fusarium* spp. in durum wheat in Algeria is still limited. This study aimed to: 1) assess the pathogenicity profiles of *Fusarium* isolates in three durum wheat cultivars (*Triticum durum* L.) using *in vitro* and *in vivo* tests; 2) assess mycotoxin levels in cultures of toxigenic *Fusarium* spp. as well as in several durum wheat grain samples using ELISA kits and LC-MS/MS methods; and 3) determine the relationship between pathogenicity and mycotoxin production.

3.3. Material and methods

3.3.1. Plant material

Pathogenicity of the *Fusarium* strain was investigated on three major wheat (*Triticum durum* L.) cultivars grown in the departments of north-eastern Algeria, namely GTAdur, Cirta, and Waha. The seeds used in this experiment were generously given by Setif's National Center for Certification and Control of Seeds and Plants (CNCC). Previously, the germination capability was determined after 1 minute of superficial sterilization with 1% sodium hypochlorite (NaClO), followed by three successive rinses with sterile distilled water and incubation on sterile filter paper moistened in Petri dishes at 25°C for 8 days. The germinated seeds were counted, and the three varieties' germination rates proved to be optimal, ranged between 90 and 100%. The mycotoxin determination was performed on 11 samples of grains retrieved from the ears of six durum wheat varieties displaying symptoms of Fusarium wilt, collected from several

states in Algeria's north-east. Wheat grains samples weighing 50 to 250 g were milled to a particle size of 0.1 mm by an MFC-90D 16 microhammer mill (Culatti, Zurich, Switzerland). The ground flour is stored in plastic bags at room temperature until analyzed.

3.3.2. Fungal material

Eighteen *Fusarium* isolates were isolated from the FHB-symptomatic grain samples of durum wheat and ears collected from various north-eastern provinces of Algeria. The set of isolates was taxonomically identified in previous chapter 1 and is codified as follows: *F. clavum* (FusBi8, FusBi1, FusBo25, FusBo28, FusBo49, FusBi2), *F. culmorum* (FusBo50, FusBo59), *F. microconidium* (FusBo26), *F. avenaceum* (FusBi7, FusBi21), *F. tricinctum* (FusBi6), *F. solani* (FusBo35), and *F. acuminatum* (FusBi23, FusBo33, FusBi15, FusBo11.5, FusBo6.12). The pathogenicity of *Fusarium* isolates was estimated through their potential for inducing symptoms (efficiency of infection, severity of disease) in addition to their ability to produce host necrosis-inducing mycotoxins (Pariaud *et al*., 2009).

3.3.3. Reagents and Chemicals

3.3.3.1. ELISA tests

Methanol and ethanol (MeOH and EtOH Carlo Erba Reagents) and water purified by the Milli-Q purification system (Millipore Corporation, Bedford, MA, USA) were used for samples preparation. Three commercial ELISA kits were provided by MyBiosource and used: (i) ZEA (Zearalenone) ELISA Kit (cat. No. MBS2548744, USA); (ii) Deoxynivalenol (DON) ELISA Kit (cat. No. MBS283277, USA); and (iii) T-2 toxin (T-2) ELISA Kit (Cat. No. MBS920908, USA).

3.3.3.2. LC-MS/MS analysis

The standards of aflatoxin-B1 (AFB1), aflatoxin-B2 (AFB2), aflatoxin-G1 (AFG1), aflatoxin-G2 (AFG2), HT-2 toxin (HT-2), T-2 toxin (T-2), fumonisin-B1 (FB1), deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), zearalenone (ZEA) and ochratoxin A (OTA), all with purity > 98%, were purchased from Sigma (West Chester, PA, USA) and Fluka (West Chester, PA, USA). The Internal Standard (IS) Ochratoxin A-(phenyl-d5) (OTA-d5) with a purity of 95% was purchased from Fluka (West Chester, PA, USA). Acetonitrile (MeCN), Methanol (MeOH), and acetic and formic acids were all HPLC-grade from Merck (Darmstadt, Germany). Ammonium acetate (analytical grade) was also from Merck.

Analytical-grade solvents, dichloromethane, and ethyl acetate were purchased from Sigma-Aldrich. Ultrapure water, purified by a Milli-Q gradient system from Millipore (Milford, MA, USA), was used to prepare the mobile phase. Anhydrous magnesium sulfate ($MgSO_4$) was purchased from Sigma-Aldrich, and sodium chloride (NaCl) was purchased from VWR, both treated at 500°C for 5h before use. Octadecylsilica (C18, particle size 55–105mm) was purchased from Waters (Milford, MA, USA), and Z-sep$^+$ was purchased from Supelco (Bellefonte, PA, USA). Standard stock solutions of 10 mg. L^{-1} of each mycotoxin were prepared in MeOH, and from these two working solutions, 1000 µg. L^{-1} each were prepared in MeOH. A stock solution of IS, d5-OTA, at 10 mg. L^{-1} was prepared in dimethylsulfoxide (DMSO), and a work solution at 500 µg. L^{-1} was prepared in MeOH. All standard solutions were kept at -18°C if not in use.

3.3.4. Instrument and analytical conditions

The high-performance liquid chromatography was performed using an HPLC system Waters Alliance 2695 (Waters, Milford) with a triple quadrupole mass spectrometer, Quattro Micro (Waters, Manchester, UK). A Kinetex C18 2.6µm particle size analytical column (150 × 4.6mm) with pre-column from Phenomenex (Tecnocroma, Portugal), maintained at 30°C, was used for chromatographic separation, as previously described by Cunha et al. (2018). The mobile phase was water/methanol/acetic acid [94:5:1 (v/v) and 5mM ammonium acetate] (solvent A) and methanol/water/acetic acid [97:2:1 (v/v)] (solvent B). The elution was conducted in a gradient that started at 95% of phase A with a linear decrease to 35% in 7 min. Then, the mobile phase A decreased to 25% at 11 min., decreased to 0% at 13 min., and remained constant until 25 min. Initial column conditions were reached at 25 min. and remained for 2 min. until the next injection. The flow rate was 0.3 mL.min$^{-1,}$ and the injection volume was 20 µL. The optimized MS/MS parameters for each analysis are listed in Table 3.1. The MS/MS acquisition was operated in positive-ion mode with multiple reactions monitoring (MRM), and the collision gas was Argon 99.995% (Gasin, Portugal) with a pressure of 2.9×10^{-3} mbar in the collision cell. Capillary voltages of 3.0 kV were used in the positive ionization mode. Nitrogen was used as desolvation and cone gas, with flows of 350 and 60 L. h^{-1}, respectively. The desolvation temperature was set to 350°C and the source temperature to 150°C. Dwell times of 0. s/scan were selected. The data were collected using the software program MassLynx 4.1.

Table 3.1. Optimized parameters for mycotoxins analysis by LC-MS/MS.

Mycotoxin/ metabolite	Retention time (min)	Parent ion (m/z)	Product ions (m/z)	Cone energy (V)	Collision energy (V)
15-ADON	8.77	339.1 $[M+H]^+$	137.1*	22	13
			321.2		
3-ADON	8.77	339.2 $[M+H]^+$	203.2	21	13
			231.2*	23	
AFG2	9.03	330.8 $[M+H]^+$	245.3	35	30
			313.1*		24
AFG1	9.37	329.0 $[M+H]^+$	243.0*	35	30
			311.2		
DON	9.60	297.0 $[M+H]^+$	203.3*	22	13
			249.0	20	11
AFB2	9.89	315.0 $[M+H]^+$	259.2*	40	33
			287.3		35
AFB1	10.32	313.0 $[M+H]^+$	241.2*	45	30
			285.2		
FB1	16.30	722.5 $[M+H]^+$	334.2*	46	40
			352.4	44	36
HT-2	16.31	442.1 $[M+H]^+$	215.3	18	15
			263.2*		
T-2	16.79	484.0 $[M+H]^+$	214.9*	21	18
			245.2	23	15
			305.2		
OTA	17.16	404.0 $[M+H]^+$	239.1*	30	20
			358.1	28	16
ZEA	17.19	319.2 $[M+H]^+$	187.0*	20	18
			283.3		16
OTA-d5	17.50	409.0 $[M+H]^+$	239.4*	32	22
			257.1		

* - Quantification ion.

3.3.5. Pathogenicity of *Fusarium* isolates

The pathogenicity of the *Fusarium* isolates was evaluated in both *in vitro* and *in vivo* tests. The first one was performed to investigate the impact on coleoptile and root growth by seed inoculation, and the second one was used to determine aggressiveness on the crown by soil inoculation.

3.3.5.1. Pathogenicity towards wheat seedlings

A pathogenicity assay was performed using eight strains, which are: FusBi7, FusBi21, FusBi15, FusBi23, FusBi8, FusBi1, FusBi2, and FusBi6. Durum wheat seeds from each cultivar were surface sterilized for 8 min. in 2% NaClO, rinsed six times in sterile distilled water, and dried. A set of five healthy wheat seeds from three cultivars were each inoculated with a 5 mm diameter fungal plug taken from a 7-day-old culture and a blank potato sucrose agar (PSA) disc (as a control). Three replicates were set up for all combinations of *Fusarium* isolate and wheat variety. The inoculated seeds were placed on sterile double-layer filter paper soaked with potato dextrose broth (PDB) in Petri dishes. To favor fungal growth, all Petri dishes were hermetically sealed with parafilm strips to maintain high relative humidity and then incubated at 25°C for six days. After that, pathogenicity was attempted by determining the coleoptile length (CL) and root system length (RSL) as well as the seminal root number (SRN), germination rate (GR), and severity of attack through the symptoms developed.

3.3.5.2. *In vivo* pathogenicity

The same *Fusarium* strains previously used in the *in vitro* pathogenicity test were studied *in vivo* according to the method described by Demirci and Dane (2003). Healthy wheat seedlings of three varieties were sown in plastic pots (12 x 10 cm, diam. by depth) containing a combination of soil and peat in a ratio of 2:1 (v/v). Each pot was sown with four surface-disinfecting wheat seeds and maintained in the greenhouse at its natural temperature and photoperiod. The artificial infection was obtained through the direct contact of a mycelial explant 5 mm in diameter from a 7-day-old *Fusarium* colony with each seed, followed by its cover with a thin layer of soil mixture 2 cm in height. Control seeds were similarly inoculated with only an agar plug without fungus. The pots and later the plants were watered frequently, depending on the soil moisture and when needed. The experimental pattern adopted was a randomized complete block design with three replicates (pots) per variety, each with four seeds for each pathogen. Fifty days post inoculation (DPI), three seedlings were carefully removed from the soil of each pot and thoroughly washed to get rid of all adhering soil particles so as not to mask root symptoms and influence the weight of the root system. The fourth plant of

each *Fusarium* strain versus durum wheat variety combination was allowed to complete its development cycle to full maturity to serve as a source for isolating *Fusarium* strains from the ears produced.

The well-washed plants were placed on a sterile paper towel to remove excess water, and thus the length of the root and vegetative systems (longest root, longest leaf) were measured, as well as their fresh weight. Later, Koch's postulate was performed by comparing the morphological characteristics of *Fusarium* strains re-isolated from symptomatic plants (root and crown) with those of the original inoculated isolates.

3.3.6. Mycotoxin production ability of *Fusarium* isolates in culture medium

3.3.6.1. Determination by ELISA Kit

A set of eighteen *Fusarium* isolates were tested for their ability to produce zearalenone (ZEA), T-2 toxin (T-2), and deoxynivalenol (DON). Each *Fusarium* strain was grown on potato dextrose agar (PDA) medium in the dark at 25°C for 15 days (Noorabadi *et al.*, 2021). Then, ELISA kits were used for the analysis of DON (cat. No. MBS283277), ZEA (cat. No. MBS2548744), and T-2 (cat. No. MBS920908). Mycotoxin extraction and determination for each immunoassay kit were performed according to the manufacturer's instructions, and the intensity of the resulting yellow color was measured using a 96-well microplate absorbance reader set to 450/630 nm (T-2, DON) and 450 nm (ZEA). Calibration curves for the quantification of DON, ZEA, and T-2 were performed with the OD values of the standard concentration established for each kit (Table 3.2). The concentration range of T-2, DON, and ZEA can be obtained by comparing the average OD value of the sample with that of the standard solution, and sample concentrations in each toxin were calculated according to the manufacturer's instructions using the Microsoft Excel program. The correlation coefficient (R^2) of the calibration curve ranged between 0.990 and 1.000. Limits of detection (LODs) were 6 $\mu g.kg^{-1}$ (ZEA), 150 $\mu g.kg^{-1}$ (DON), and 30 $\mu g.kg^{-1}$ (T-2).

Table 3.2. Standard concentrations used in mycotoxin dosage analysis.

Mycotoxin	Concentration	C1	C2	C3	C4	C5	C6
DON	$\mu g.kg^{-1}$	0.0	3.0	9.0	27.0	81.0	243.0
ZEA	$\mu g.kg^{-1}$	0.0	0.3	0.9	2.7	8.1	24.3
T-2	$\mu g.kg^{-1}$	0.0	0.3	0.9	2.7	8.1	

3.3.6.2. Determination by LC-MS/MS technique

LC-MS/MS analysis was also performed to determine the typology of the mycotoxins produced by *Fusarium* strains in this case: DON, 15-ADON, 3-ADON, and ZEA. The test was performed in the Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Portugal, and was applied to a set of six isolates, including FusBi1, FusBi6, FusBi7, FusBo26, FusBo33, and FusBo59. Mycotoxin ability was performed using PDA medium. Three pieces of a previous 7-days sabouraud dextrose agar (SAB) subculture were inoculated into PDA and were incubated at 25ºC during 21 days (Samson, 2010). After 21 days, the medium was lyophilised for further extraction and mycotoxins determination.

Mycotoxins were extracted according to Smedsgaard (1997), with some modifications. Briefly, 0.5 g of dried culture was weighed into a 50 mL centrifuge tube, and 40 µL of OTA-d5 (IS) at 500 µg. L^{-1} was added. After leaving the samples overnight for equilibration, 10 mL of water was added and shaken for 30 min. After that, 10 mL of methanol, dichloromethane, and ethyl acetate (1:2:3, vol/vol/vol) (HPLC-grade purity) containing 1% formic acid were homogenized for 1 min. and sonicated for 15 min. The tubes were then centrifuged at 4000 g for 15 min. to induce phase separation and mycotoxins partitioning. Then, the upper layer was transferred to an injection vial and evaporated to dryness under a stream of nitrogen (SBH CONC/1 sample concentrator from Stuart®; Staffordshire, OSA, USA). The final extract was reconstituted in 150 µL of mobile phase B (methanol: water: acetic acid (97:2:1) with 5 mM ammonium acetate) and transferred to a 200 µL insert glass for LC-MS/MS analysis. Each sample was injected twice.

3.3.7. Mycotoxin analysis in wheat grains

3.3.7.1. Determination by LC-MS/MS technique

Mycotoxins extractions were performed by the QuEChERS (quick, easy, cheap, effective, rugged and safe) method with some modifications (Cunha *et al.*, 2018). Briefly, 5.0 g of grounded sample was weighed into a 50 mL centrifuge tube, and 200 µL of OTA-d5 (IS) at 500 µg. L^{-1} was added. After leaving the samples overnight for equilibration, 5 mL of water was added and shaken for 30 min. After that, 5 mL of MeCN (HPLC purity) with 1% formic acid was added along with 2.0 g of $MgSO_4$ anhydrous salt and 1.0 g of NaCl, and tubes were mixed for 1 h in an orbital shaker. The tubes were then centrifuged at 4000 g for 15 min. to induce phase separation and mycotoxins partitioning. For the dSPE clean-up procedure, exactly 1.2 mL of the organic phase was transferred to a 4 mL vial containing 100 mg C18 and 50 mg Z-sep$^+$, homogenized for 30 s, and centrifuged for 4000 g for 5 min. Then, 0.80 mL from the

upper layer was transferred to an injection vial and evaporated to dryness under a stream of nitrogen (SBH CONC/1 sample concentrator from Stuart®; Staffordshire, OSA, USA). The final extract was reconstituted in 750 µL of mobile phase B (methanol: water: acetic acid (97:2:1) with 5 mM ammonium acetate) and transferred to a 2 mL glass vial for LC-MS/MS analysis. Each sample was injected twice.

3.3.8. Statistical analysis

To determine the importance of pathogenicity on various types of wheat between isolated *Fusarium* species, a statistical analysis (ANOVA) was carried out using the SPSS V. 25 software package (SPSS, 2017) at a probability threshold of 5%. To reflect the measurement variability, the results are reported as the mean ± SEM (standard error of the mean), and homogenous groups are identified using Tukey's HSD test. Additionally, Pearson correlation tests were used to examine the connections between the parameters evaluated for pathogenicity and the mycotoxin levels.

3.4. Results

3.4.1. Pathogenicity of *Fusarium* isolates towards wheat seedlings

The susceptibility of the three most commonly sown durum wheat varieties in the study area was assessed, along with the pathogenicity of isolated *Fusarium* strains, by measuring various developmental parameters such as coleoptile and root system length, number of seminal roots, germination rate, and severity via symptom induction (Figure 3.1).

According to the statistical analysis of the results, the eight *Fusarium* isolates had a negative and significant ($p<0.01$) impact on the length of the coleoptiles (CL), the length of the root system (RSL), the number of seminal roots (SRN), and the germination rate (GR) (Table 3.3). However, the statistical analysis revealed no significant difference between the three durum wheat cultivars and the eight isolates regarding the growth parameters investigated. In terms of coleoptile length, the FusBi7 and FusBi21 strains significantly lowered the CLs of the three varieties evaluated when compared to their respective controls. When infected with the FusBi7 strain, coleoptile lengths measured 1.57, 0.3, and 0.11 cm for the Cirta, Waha, and GTAdur varieties, respectively. In contrast to these findings, the lengths of the FusBi1 and FusBi2 strains were extremely close to those of the controls (Table 3.3).

Regarding the effect of *Fusarium* strains on root system length, the FusBi7 strain is the most aggressive, yielding lengths of 2.21, 1.09, and 0.62 cm for the Cirta, Waha, and GTAdur

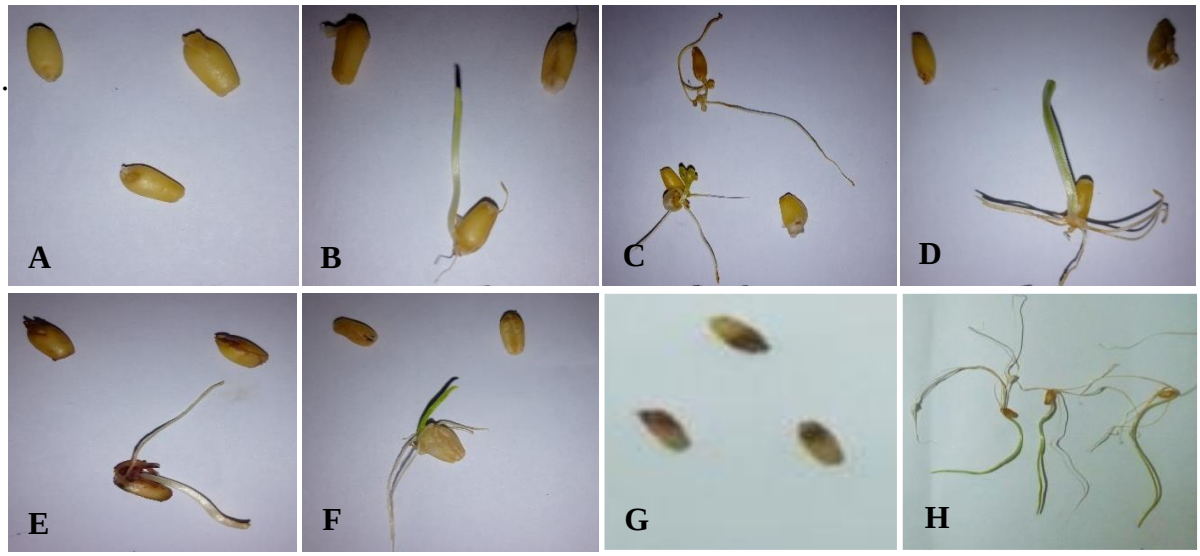


Figure 3.1. Symptoms on seedlings of durum wheat cv "GTA dur" inoculated with mycelial plugs of *Fusarium* spp. after 6 days. **A-C.** *F.clavum* (FusBi1, FusBi2, and FusBi8). **D-E.** *F. acuminatum* (FusBi15, FusBi23). **F.** *F.tricinctum* (FusBi6). **G.** *F.avenaceum* (FusBi7). **H.** Control.

varieties, respectively. This is much lower than the 8.89, 8.36, and 9.14 cm achieved with their respective controls. Furthermore, the FusBi7 strain had the greatest impact on the number of seminal roots, once again demonstrating its aggressiveness and yielding an average of 0.53 for GTAdur, 1.87 for Waha, and 2.8 for Cirta. This is significantly lower than the 5.50, 5.43, and 5.97 cm control values for the same varieties (Table 3.3).

Regarding the influence of *Fusarium* isolates on the germination of durum wheat seeds, the FusBi7 strain caused a significant drop, reaching 20% with the GTAdur variety. Concerning average germination rates, the GTAdur appears to be the most susceptible to diseases, with a germination rate of 73.33%, followed by the Waha variety (87.5%), and lastly, the Cirta variety (93.33%).

Finally, in what symptom induction is concerned, we note the emergence of root necrosis on both the coleoptile and the seminal roots (Figure 3.1), which rarely results in the death of the infected plant. The estimated severity generated by the strains examined reveals that the FusBi7 strain is the most severe of the three studied varieties, with rates of 0.3, 0.53, and 0.67 for the Cirta, Waha, and GTAdur, respectively. This is supported by the short coleoptile lengths observed in the same types when infected with the FusBi21 strain, which resulted in a high severity rate due to root coleoptile necrosis symptoms (Table 3.3).

Table 3.3. Mean of *in vitro* pathogenicity on germination parameters.

Fusarium Strains	Coleoptile length (CL) (cm)			Root system length (RSL) (cm)			Seminal root number (SRN)			Germination rate (GR) (%)			Severity		
	Cirta	**GTAdur**	**Waha**	**Cirta**	**GTAdur**	**Waha**	**Cirta**	**GTAdur**	**Waha**	**Cirta**	**GTAdur**	**Waha**	**Cirta**	**GTAdur**	**Waha**
Control	6.540^{c}	6.350^{c}	6.117^{bc}	8.890^{c}	9.140^{c}	8.360^{c}	5.967^{b}	5.500^{b}	5.4333^{bc}	100.000^{a}	100.000^{b}	100.000^{a}	0.000^{a}	0.000^{a}	0.000^{a}
FusBi1	5.900^{c}	5.427^{c}	6.280^{c}	6.813^{c}	3.640^{abc}	6.413^{bc}	6.000^{b}	4.333^{b}	5.5333^{bc}	100.000^{a}	80.000^{ab}	93.333^{a}	0.400^{a}	0.133^{a}	0.333^{a}
FusBi15	3.480^{ab}	3.833^{abc}	3.467^{b}	9.267^{c}	7.5133^{bc}	7.587^{c}	5.600^{b}	4.933^{b}	5.0667^{bc}	100.000^{a}	86.667^{ab}	86.667^{a}	0.200^{a}	0.000^{a}	0.267^{a}
FusBi2	6.467^{c}	5.327^{c}	5.567^{bc}	8.807^{c}	6.860^{abc}	7.773^{c}	4.200^{ab}	4.067^{ab}	5.1333^{bc}	100.000^{a}	73.333^{ab}	86.667^{a}	0.267^{a}	0.133^{a}	0.133^{a}
FusBi21	2.127^{a}	1.000^{ab}	0.840^{a}	6.680^{c}	3.633^{abc}	3.713^{ab}	3.933^{ab}	3.40^{ab}	3.400^{ab}	100.000^{a}	93.333^{b}	86.667^{a}	0.467^{a}	0.533^{a}	0.533^{a}
FusBi23	3.773^{ab}	3.400^{abc}	4.533^{bc}	3.033^{a}	2.1400^{ab}	3.933^{ab}	5.467^{b}	2.933^{ab}	5.2667^{bc}	86.667^{a}	66.667^{ab}	93.333^{a}	0.133^{a}	0.733^{a}	0.133^{a}
FusBi6	4.993^{bc}	4.233^{bc}	5.980^{bc}	8.987^{c}	6.633^{abc}	7.740^{c}	2.800^{a}	4.467^{b}	5.800^{c}	100.000^{a}	86.667^{ab}	100.000^{a}	0.133^{a}	0.200^{a}	0.200^{a}
FusBi7	1.573^{a}	0.113^{a}	0.300^{a}	2.213^{a}	0.620^{a}	1.087^{a}	4.267^{ab}	0.533^{a}	1.867^{a}	80.000^{a}	20.000^{a}	60.000^{a}	0.300^{a}	0.667^{a}	0.533^{a}
FusBi8	3.700^{ab}	4.687^{bc}	5.667^{bc}	4.573^{ab}	4.693^{abc}	7.840^{c}	6.200^{b}	3.667^{ab}	5.2667^{bc}	80.000^{a}	80.000^{ab}	93.333^{a}	0.267^{a}	0.133^{a}	0.467^{a}
Mean	4.002	3.503	4.079	6.297	4.467	5.761	4.808	3.542	4.667	93.333	73.333	87.500	0.271	0.317	0.325

Different lowercase letters in the same column indicate significant differences analysed by ANOVA followed by Tukey's HSD test ($p \leq 0.05$). Data are mean ($n = 3$).

3.4.2. Pathogenicity of *Fusarium* isolates *in vivo*

The healthy wheat seedlings were injected with the mycelium of eight isolates to determine their pathogenicity, showed fifty days post-inoculation, same symptoms occurred with the initial symptoms reported (Figures 3.2b, d and f). There were no symptoms in the control plants (Figures 3.2c, e and g). The pathogenic fungi were re-isolated from damaged plants (root and crown), and their identity was confirmed morphologically when compared to the isolates used as inoculum, demonstrating that Koch's postulates were fulfilled. There was no re-isolation of a related fungus from the control plants. The findings of the vegetative system weight (VSW), root system length (RSL), and root system weight (RSW) parameters show a highly significant difference ($p<0.001$) in the pathogenicity of *Fusarium* strains and a significant difference ($p<0.05$) in the vegetative system length (VSL).

In vivo pathogenicity data demonstrate that some strains had a detrimental impact on the development parameters tested, including root system length and weight and vegetative system length and weight. In contrast, others provided values remarkably comparable to those reported by control plants. In terms of the effect on the length of the vegetative system, all three cultivars were susceptible to a specific *Fusarium* strain. Thus, the FusBi7 isolate produced the lowest VSL value of 24.89 cm for the Cirta variety, FusBi15 produced 21.11 cm for the GTAdur, and FusBi1 produced 15.83 cm for the Waha variety, all of which are lower than the 34.33, 32.33, and 34.16 VSL values of control plants (Table 3.4). According to the findings, the effects on the length of the vegetative system were translated into the weight of the vegetative system. Thus, FusBi7 reduced the VSW on the Cirta variety to 1.19 g, FusBi15 created 1.21 g on the GTAdur variety, and FusBi1 produced 0.43 g on the Waha variety. These VSW weights are much lower than the 1.92, 1.76, and 1.66 g reported with the control plants for the same kinds (Table 3.4).

In terms of the impact on the root system, the FusBi15 strain caused the shortest length of the GTAdur variety's root system at 13.89 cm, while the FusBi7 strain lowered this length to 17.33 cm and 16.11 cm for the Cirta and Waha varieties, respectively. When compared to the control plants, these strains had considerably ($p<0.01$) lower RSL values (28.61, 27.77, and 27.88 cm for the same varieties, respectively) (Table 3.4). However, in absolute terms, the FusBi1 strain produced the lowest RSW of 0.28 g on the Waha variety, while FusBi6 produced 0.44 g on Cirta and FusBi15 produced 0.47 g on the GTAdur type. When compared to the control, these strains had considerably ($p<0.01$) lower RSW values compared to the control plants, 0.98, 0.97, and 1.18 g for the same varieties, respectively (Table 3.4).

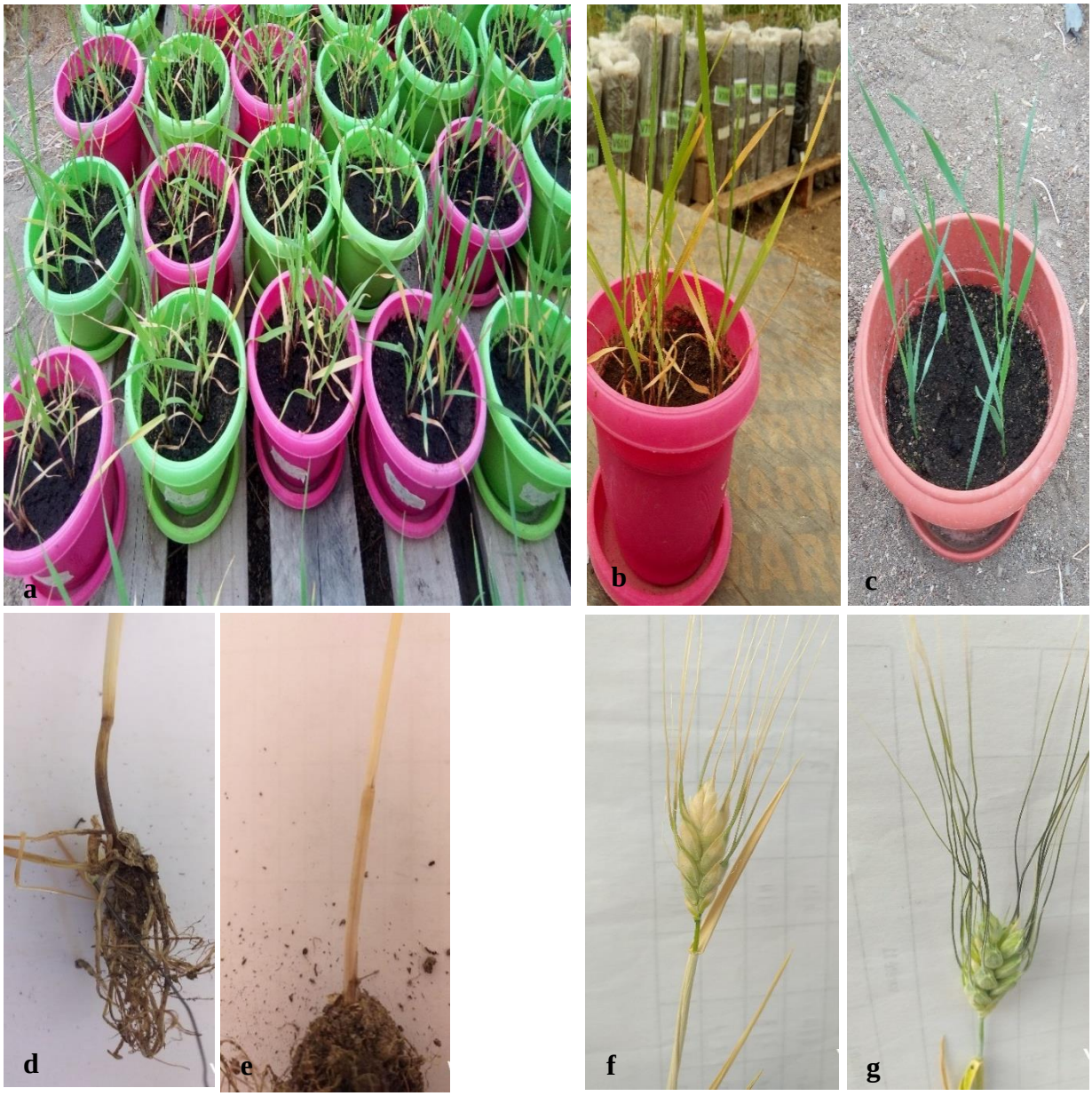


Figure 3.2. Pathogenicity test on seedlings durum wheat of three varieties inoculated with *Fusarium* spp. **a-b.** Diseased plants in the greenhouse; **c.** Control plants; **d**. Crown browning symptoms (plant of GTAdur variety inoculated with *F. avenaceum* (FusBi7); **e.** Crown asymptomatic of control plant; **f.** Infected head (plant of Waha variety inoculated with *F. clavum* (FusBi1); **g.** Head asymptomatic of control plant (50 DPI).

Table 3.4. Mean of *in vivo* pathogenicity.

Fusarium Strains	Vegetative system length VSL (cm)			Vegetative system weight VSW (g)			Root system length RSL (cm)			Root system weight RSW (g)		
	Cirta	**GTAdur**	**Waha**	**Cirta**	**GTAdur**	**Waha**	**Cirta**	**GTAdur**	**Waha**	**Cirta**	**GTAdur**	**Waha**
Control	34.333^{c}	32.333^{b}	34.167^{c}	1.9167^{ab}	1.762^{a}	1.658^{d}	27.778^{b}	28.611^{bc}	27.889^{a}	0.977^{bc}	1.188^{bc}	0.980^{b}
FusBi1	33.000^{bc}	28.833^{ab}	15.833^{a}	1.669^{ab}	1.377^{a}	0.434^{a}	26.833^{ab}	27.333^{bc}	19.583^{a}	0.811^{abc}	0.861^{abc}	0.282^{a}
FusBi15	34.889^{c}	21.111^{a}	31.111^{ab}	2.087^{b}	1.257^{a}	1.451^{cd}	22.333^{ab}	13.889^{a}	21.556^{a}	1.158^{cd}	0.471^{a}	0.727^{ab}
FusBi2	35.167^{c}	30.333^{ab}	19.000^{ab}	1.872^{ab}	1.444^{a}	0.572^{ab}	25.500^{ab}	26.667^{bc}	14.833^{a}	1.454^{d}	1.106^{bc}	0.332^{a}
FusBi21	30.583^{abc}	28.167^{ab}	31.167^{ab}	1.248^{a}	1.210^{a}	1.256^{bcd}	24.667^{ab}	29.833^{c}	29.278^{a}	0.696^{abc}	1.129^{bc}	1.012^{b}
FusBi23	35.667^{c}	27.222^{ab}	30.889^{ab}	1.836^{ab}	1.619^{a}	1.445^{cd}	20.778^{ab}	19.556^{ab}	20.000^{a}	0.884^{abc}	0.749^{ab}	0.697^{ab}
FusBi6	26.333^{ab}	31.667^{ab}	27.500^{ab}	1.376^{ab}	1.341^{a}	1.2556^{bcd}	24.667^{ab}	29.167^{bc}	29.500^{a}	0.439^{a}	0.659^{ab}	0.642^{ab}
FusBi7	24.889^{a}	28.111^{ab}	24.111^{ab}	1.193^{a}	1.642^{a}	0.908^{abcd}	17.333^{a}	21.444^{abc}	16.111^{a}	0.498^{ab}	0.837^{abc}	0.418^{ab}
FusBi8	29.833^{abc}	31.333^{ab}	25.833^{ab}	1.341^{ab}	1.583^{a}	0.724^{abc}	28.500^{b}	29.167^{bc}	25.667^{a}	0.867^{abc}	1.426^{c}	0.639^{ab}
Mean	31.295	28.347	25.681	1.578	1.434	1.006	23.826	24.632	22.066	0.851	0.905	0.594

Different lowercase letters in the same column indicate significant differences analysed by ANOVA followed by Tukey's HSD test ($p \leq 0.05$). Data are mean ($n = 3$).

Based on the *in vivo* pathogenicity results, the genotypic responses to pathogenic isolates show varied behavior and a substantial difference at the 5% threshold. It appears that the Cirta variety's vegetative development parameters are least significantly influenced, followed by GTAdur and, finally, Waha. They are represented by a VSL of 31.29, 28.34, and 25.68 cm and a VSW of 1.57, 1.43, and 1.00 g, respectively. However, no significant variation was seen at the 5% threshold in the parameters linked to the length and weight of the root system (Table 3.4).

3.4.3. Mycotoxin production ability of *Fusarium* isolates in culture medium

Fusarium isolates' mycotoxigenic potential was evaluated using two analytical techniques: Kit ELISA and LC-MS/MS. All examined strains could produce the three categories of mycotoxins evaluated using serological methods. Because of the technique's sensitivity, the content of toxins detected by LC-MS/MS is significantly higher. The mycotoxins screening by ELISA revealed that the FusBo59 and FusBi15 strains have the highest ZEA production levels of 3.941 and 3.116 $\mu g.kg^{-1}$, respectively (Table 3.5). In contrast, the FusBo59 strain emerged as the most toxigenic for DON, with a high level equal to 7.128 $\mu g.kg^{-1}$ in the ELISA test and 373196.19 $\mu g.kg^{-1}$ by LC-MS/MS. The amounts of the third mycotoxin tested, T-2, ranged from 0.281 to 0.349 $\mu g.kg^{-1}$ without significant variation.

The LC-MS/MS study revealed that all of the strains investigated produced DON in concentrations ranging from 6.41 to 373196.19 $\mu g.kg^{1}$ (Table 3.6). Notably, only two strains, FusBo33 and FusBo59, exhibited remarkable 15-ADON production, reaching 8.62 and 2090.24 $\mu g.kg^{-1}$, respectively. In contrast, only one strain of FusBo26, on the other hand, could produce ZEA (27.63 $\mu g.kg^{-1}$). Additionally, for the production of 3-ADON toxin, a single strain, FusBi6, synthesized the mycotoxin; however, the amount was smaller than the LOQ. It should be noted that the interday precision was estimated using the relative standard deviation (RSD) of the two weeks apart data. The data show an overall percent RSD range of 1 to 14%, well within acceptable variability limits.

3.4.4. Mycotoxin analysis in wheat grains

The LC-MS/MS analysis of the durum wheat samples from which the various mycotoxigenic *Fusarium* spp. were isolated reveals a significant diversity in terms of mycotoxin type and quantity (Table 3.7). Thus, in W50 and W59 durum wheat samples, levels of DON of 33.51 and 624.96 $\mu g.kg^{-1}$ and 15-ADON of 2.45 and 18.14 $\mu g.kg^{-1}$ were detected in addition to ZEA (2.22 $\mu g.kg^{-1}$) and 3-ADON (61.18 $\mu g.kg^{-1}$) in the wheat sample W59. ZEA was also detected in sample W26 (1.82 $\mu g.kg^{-1}$).

Table 3.5. Mycotoxins detection by ELISA in the culture extracts.

Fusarium isolates	ELISA		
	ZEA (µg.kg^{-1})	DON (µg.kg^{-1})	T-2 (µg.kg^{-1})
FusBi1	0.501	0.338	0.305
FusBi15	3.116	0.100	0.281
FusBi2	0.297	0.249	0.305
FusBi21	0.634	0.125	0.320
FusBi23	0.463	0.043	0.320
FusBi6	0.200	0.063	0.305
FusBi7	0.429	0.014	0.305
FusBi8	0.501	0.093	0.320
FusBo26	0.366	0.125	0.349
FusBo33	0.418	0.043	0.349
FusBo59	3.941	7.128	0.320
FusBo6.12	0,501	0,313	0,334
FusBo28	0,542	0,079	0,305
FusBo25	0,376	0,002	0,349
FusBo50	0,571	0,1	0,38
FusBo11.5	0,356	0,05	0,364
FusBo35	0,297	0,29	0,364
FusBo49	0,161	0,013	0,397

Table 3.6. Mycotoxins detection by LC-MS/MS in the culture extracts.

	DON		15-ADON		3-ADON		ZEA	
Fusarium isolates	Average µg.kg^{-1}	RSD %	Average µg.kg^{-1}	RSD %	Average µg.kg^{-1}	RSD %	Average µg.kg^{-1}g	RSD %
FusBi1	34.57	13	-		-		-	
FusBi6	6.41	6	-		<LOQ		-	
FusBi7	34.39	4	-		-		-	
FusBo26	62.08	6	-		-		27.63	4
FusBo33	102.61	14	8.62	3	-		-	
FusBo59	373196.19	3	2090.24	1	-		-	

LOQ=2.5 µg.kg^{-1}; RSD%: relative standard deviation.

"-" not detected.

Table 3.7. Types and levels of mycotoxins detected by LC-MS/MS in cereal samples.

Positive	DON		15-ADON		3-ADON		AFG2		AFB2		T-2		ZEA	
Samples code	Average µg.kg^{-1}	RSD %	Average µg.kg^{-1}	RSD %	Average µg.kg^{-1}	RSD %	Average µg.kg^{-1}	RSD %	Average µg.kg^{-1}	RSD %	Average µg.kg^{-1}	RSD %	Average µg.kg^{-1}	RSD %
E39	-		8.49	12	-		2.30	0	-		-		<LOQ	
E44	-		11.11	8	-		-		-		-		-	
E49	-		-		-		-		16.28	7	-		-	
E47	-		-		-		-		20.82	15	-		-	
W26	-		<LOQ		-		-		-		-		1.82	2
W59	624.96	1	18.14	2	61.18	5	-		-		-		2.22	15
W50	33.51	1	2.45	8	-		-		-		-		-	
E54	-		7.98	4	-		-		-		-		-	
E55	-		-		-		1.04	5	-		-		-	
E40	-		-		-		-		-		<LOQ		-	
W33	-		1.72	2	-		-		-		-		-	

LOQ=1 µg.kg^{-1}; RSD%: relative standard deviation.

"-" not detected.

Furthermore, 15-ADON was found to be the most common mycotoxin type in durum wheat grain samples, with six samples having values above the LOQs. AFG2 toxin, on the other hand, was identified in two samples E55 and E39 (1.04 μg.kg^{-1} and 2.30 μg.kg^{-1} respectively), and AFB2 in two samples, E49 and E47, with values of 16.28 and 20.82 μg.kg^{-1}, respectively. Finally, T-2 was found in only one sample, E40, but the value was less than the LOQ. It should be noted that the interday precision was estimated using the relative standard deviation (RSD) of the two weeks apart data. The results show an overall percent RSD ranging from 1 to 15%, which falls within the acceptable variability limits.

3.4.5. Correlation between pathogenicity and mycotoxins production

The correlation matrix analysis of plant and seed development parameters tested *in vitro* and *in vivo* indicated significant positive associations. Thus, the coleoptile length is proportional to the number of seminal roots, and the germination rate is positively correlated with the length of the root system and the number of seminal roots. The severity of *Fusarium* strains is negatively related to the length of the coleoptile and root system and the number of seminal roots (Table 3. 8).

Additionally, the correlation matrix between the productions of the various kinds of mycotoxins reveals that the production of the three categories of toxins is negatively connected; this finding is more prominent with ZEA and T-2. The more ZEA produced, the less T-2 is present in the examined sample. Furthermore, the two matrices of the *in vitro* and *in vivo* pathogenicity experiments show no link between disease induction and toxin generation, each factor evolving independently of the other (Tables 3.8, 3.9).

Table 3.8. Pearson correlation matrix of *in vitro* pathogenicity on growth parameters and mycotoxin types (ELISA test).

Parameters	CL	RSL	SRN	GR	Severity	ZEA	DON	T-2
CL	1							
RSL	0.669	1						
SRN	**0.841****	**0.876****	1					
GR	0.578	**0.771***	**0.871****	1				
Severity	**-0.816***	**-0.850****	**-0.843****	-0.541	1			
ZEA	-0.120	0.364	0.281	0.183	-0.328	1		
DON	0.614	0.405	0.551	0.430	-0.286	-0.091	1	
T-2	-0.105	-0.489	-0.333	-0.054	0.531	**-0.764***	-0.116	1

**. The correlation is significant at the 0.01 level (two-sided).
*. The correlation is significant at the 0.05 level (two-sided).

Table 3.9. Pearson correlation matrix of *in vivo* pathogenicity on growth parameters and mycotoxin types (ELISA test).

Parameters	VSL	RSL	VSW	RSW	ZEA	DON	T-2
VSL	1						
RSL	0.170	1					
VSW	0.628	-0.559	1				
RSW	0.537	0.293	-0.001	1			
ZEA	0.150	-0.406	0.571	0.070	1		
DON	-0.370	0.205	-0.429	0.173	-0.091	1	
T-2	0.342	0.488	-0.321	0.340	**-0.764***	-0.116	1

*. The correlation is significant at the 0.05 level (two-sided).

3.5. Discussions

As is well known, the pride of FHB outbreaks comes from an economic and public health standpoint. It's worth raising awareness and interest, especially given the scarcity of insights on the aggressiveness and mycotoxin patterns of *Fusarium* spp. it is occurring in Algerian wheat, to address knowledge gaps that may have a broad vision of the sanitary quality of the grains. Therefore, this study highlights the variability in aggressiveness among *Fusarium* isolates collected from the north-eastern regions of Algeria and the diversity of mycotoxins, which are implicated in plant-pathogen interactions during the infection process. Further, the correlation between pathogenicity parameters and chemotype patterns was investigated.

Our findings of seedling pathogenicity tests revealed significant variation in aggressiveness between isolates and between species, with *F. avenaceum* FusBi7 being the most aggressive and causing severe symptoms in the *in vitro* and in vivo pathogenicity tests. *In vitro*, it inhibited germination, reduced coleoptile and root system lengths, and stunted seminal root development. *In vivo*, it caused significant reductions in vegetative system length and weight, root system length, and induced crown browning symptoms. Despite our findings, Moparthi *et al.* (2021) claimed that *F. avenaceum* isolates were highly aggressive on the crown tissues of wheat seedlings. The isolate of *F. avenaceum* showing severe symptoms on crown tissues stood out as being severely aggressive on wheat head plants in south-western Ethiopia by Kebede *et al.* (2020). Recent investigations reported that *F. avenaceum* isolates were the most aggressive on wheat and diverse crops, such as chickpeas (Armstrong-Cho *et al.*, 2023) and barley (Inbaia *et al.*, 2023). However, contrary to our findings, Özer *et al.* (2020) also observed that *F. avenaceum* was able to cause crown in wheat plants. Still, it was intermediate with a 2.72 disease severity score compared to *F. culmorum* (3.52), *F. pseudograminearum* (3.49), and *F. graminearum* isolates (3.23). What's more, results reported by Fernandez and Chen (2005)

reveal that *F. avenaceum* poses only a minor concern to wheat. The differences in disease severity noted in the aforementioned studies could be attributed to several factors, including the genetic diversity of aggressiveness within the pathogenic isolates tested, differences in the tolerance of the host cultivars used, growth conditions, applied inoculation techniques and evaluation methods that need to be taken into account as well.

In the greenhouse assay, the pathogen isolates from the heads can trigger the disease crowns of wheat, which may suggest that both diseases share a common etiological agent. The dynamics of *Fusarium* inoculum leading to underground illnesses may serve as a source of inoculum driving infections in wheat heads, eventually resulting in Fusarium head blight in the next season (Fernandez and Chen, 2005).

The varietal resistance of the host cultivars was the most paramount variable affecting the severity of head and crown diseases in wheat. The examined durum wheat cultivars exhibited diverging responses to the initial infection, with Cv. Cirta being less susceptible to the pathogen infection than other genotypes tested. The results reported by Bouanaka *et al*. (2021) are similar to those of the present study, which used multiple methods to evaluate the susceptibility of durum wheat genotypes towards FCR and FHB in Algeria. They found that Cirta and GTAdur cultivars presented the highest levels of tolerance for initial seed infection to the pathogen *F. culmorum* strain FC11 with ($GI\%_{Cirta}$ = 25.51%, $AUDPC1_{Cirta}$ = 33.16%) and ($GI\%_{GTAdur}$ = 29.16%, $AUDPC1_{GTAdur}$ = 36.10%) compared to Waha cultivars with susceptibility of 38.00% and 46.50% for GI and AUDPC1, respectively.

The data of the crown inoculation assay in the greenhouse showed that Cirta and GTAdur genotypes were more tolerant to the disease than Waha genotypes, which is in close conformity with the conclusion of Bouanaka *et al*. (2021), who observed that Cirta and GTAdur cultivars were tolerant with an area under disease progress curve (AUDPC1) of 33.16% and 36.10%, respectively, while Cv. Waha were moderately sensitive, with an AUDPC1 of 46.5%. Similair finding was obtained by Bencheikh *et al*. (2018), where the Waha cultivar was noticed to be considerably more susceptible to the infection of *F. chlamydosporum*, resulting in the following reduction: 71.15% of fresh weight of vegetative system (FWVS), 75.04% of length of root system (LRS), and 82.58% of length of vegetative system (LVS).

We showed that there are significant differences in *Fusarium* strains' disease-causing ability as well as in the behavior of durum wheat genotypes. This suggests the existence of intrinsic diversity in the genetic origin of the host-pathogen interaction. Due to this diversity, the results reported here offer not only valuable insights into the aggressiveness profile of *Fusarium* isolates responsible for head blight and crown rot in wheat but also important data about the

resistance level of local durum wheat genotypes. This knowledge will be helpful for breeding programs intended to enhance cultivar resistance to decrease yield losses and mycotoxin accumulation in Algeria and worldwide.

The current occurrence of such pathogenic *Fusarium* species in durum wheat has raised further issues through their contribution to the multiple mycotoxin contamination of Algerian cereals, mitigating wheat quality and therefore threatening consumer health and agro-food systems. This situation should have raised public health awareness. Each pathogen species has its specific mycotoxigenic profile. Hence, accurate mycotoxin risk assessment is closely associated with the application of various advanced detection tools. Overall, the results clearly demonstrate that the FusBo59 strain (*F. culmorum*) has a high toxigenic ability to produce DON compared to the rest of the strains, with a maximum level equal to 7.128 $\mu g.kg^{-1}$ in the ELISA test and 373,196.19 $\mu g.kg^{-1}$ by LC-MS/MS exceeding the legal limits permitted by the EU at 1750 $\mu g.kg^{-1}$ for durum wheat intended for human and animal consumption (European Commission, 2006). Similar analysis on *F. culmorum* strains collected from various cropping areas of North Algeria showed 75% of strains were able to produce a significant content of DON that reached 80000 $\mu g.kg^{-1}$, also exceeding EU limits. DON levels in *F. culmorum* detected in this work (62239 $\mu g.kg^{-1}$ mean) were typically higher than the mean value for DON levels in wheat (12300 $\mu g.kg^{-1}$) (Miedaner *et al.*, 2021). These findings underscore the potential threat of these *Fusarium* strains to human health. Moreover, isolates of *F. culmorum* associated with FHB collected in Europe and Asia produce a new mycotoxin type A trichothecene, NX-2, simultaneously with DON, 3-ADON, or NIV (Schiwek *et al.*, 2022).

The chromatographic analysis of DON contents revealed its presence in all evaluated isolates, with significant levels ranging from 6.41 to 373196.19 $\mu g.kg^{-1}$. This finding suggests that these isolates possess an inherent ability to contaminate grain cereals with this toxin. Noteworthy, the harmfulness of DON nowadays resides not only in their detection with high contamination levels and prevalence in grain-based foods worldwide but also in the stability of these compounds during different food processing, which has attracted worldly attention through further research focusing on the application of multiple DON detoxification approaches (Feizollahi and Roopesh, 2022; Li *et al.*, 2023). Another intriguing observation is that DON was consistently the most prevalent mycotoxin detected in the study, and no culture extracts containing only DON derivatives (3-ADON and/or 15-ADON) were found. This finding aligns with the research conducted by Yan *et al*. (2020), which demonstrated that DON (95.51%) was significantly more prevalent in infected wheat than in its acetylated derivatives.

The FusBo26 isolate (*F. microconidium*) produced a mixture of DON and ZEA mycotoxins, indicating that grain-based foods are likely to be contaminated with multi-mycotoxins compounds that could have heavy impacts on human and animal health, owing to the possible antagonistic, additive, or synergic effects (Wang *et al*., 2018). In the Netherlands, the co-occurrence of DON, ZEA, enniatin B and B1, HT-2, and NIV in wheat samples has been reported (Van der Fels-Klerx *et al.,* 2021). A Brazilian survey on wheat flour reported the simultaneous occurrence of DON, ZEA, and T-2 at high concentrations and incidence (Dos Santos *et al*., 2021).

T-2 production was recovered in our study at trace levels with all the examined isolates, reaching a maximum 0.35 $\mu g.kg^{-1}$. On the contrary, a significant T-2 and HT-2 content was obtained in durum wheat grains from Southern Italy in 2014 (150 $\mu g.kg^{-1}$ mean) (Haidukowski *et al*., 2022). Additionally, T-2 has been shown to contaminate other cereals from the UK at higher values in the range of 171–1426 $\mu g.kg^{-1}$ mean HT-2 + T-2 amount in winter oat varieties (Edwards and Stancic, 2022) and 279.34 $\mu g.kg^{-1}$ in corn (Zhang *et al.,* 2021). Hence, the durum wheat in Algeria is less susceptible to T-2 contamination. The very low amounts determined in the present research are not expected to have any adverse impacts on human health or food security.

Our monitoring study emphasized the natural co-occurrence of multi-mycotoxins in Algerian durum wheat, with *Fusarium* mycotoxins being the most prevalent. In contrast, *Aspergillus* mycotoxins were detected at low levels and frequencies. Among the Fusariotoxins, DON and its acetylated derivatives (15-ADON and 3-ADON), type B trichothecenes, are incredibly intoxicating through the impact of their metabolites on the gastrointestinal tract and hematopoietic progenitor cellular systems and contribute to intestinal epithelial cell necrosis (Polak-liwiska *et al*., 2021; Gab-Allah *et al*., 2023). In our survey, 15-ADON was more prevalent in wheat grains (63.6%) than DON (18.2%) and 3-ADON (9.1%). Contrarily which was observed in Chinese wheat with a higher incidence of DON (90.8%), followed by 3-ADON (69.2%) and 15-ADON (49.4%) (Zhao *et al.,* 2021). One of the possible explanations for this finding could be the significant presence of 15-ADON-producing species in Algerian durum wheat. These discrepancies in incidence could be attributed to weather patterns and the agro-ecological regions where the samples were harvested (Liu *et al*., 2016). Besides, contrary to 15-ADON, DON occurred at a lower incidence but with high concentrations in wheat samples (329 $g.kg^{-1}$ mean), compared with those encountered in 100% of the samples of Brazilian wheat flour, with a range level from 53 to 2905 $\mu g.kg^{-1}$ (Dos Santos *et al*., 2021). Otherwise, Yan *et al*. (2020) alluded to a greater prevalence of DON in wheat samples (100%) but at a relatively

moderate level (165.87 μg.kg^{-1} mean). The main reason for the differences in DON accumulation in examined samples could be a consequence of fluctuations in the ratio between DON-producing and non-DON-producing species (Beyer *et al*., 2007), host cultivars, meteorological factors, particularly temperature and relative humidity, as well as soil type.

In the current study, 18.2% of zearalenone-contaminated wheat grains had contents below the EU limits (100 μg.kg^{-1} in raw cereals), with a mean content of 2 μg.kg^{-1}. According to an EFSA Panel on Contaminants in the Food Chain survey, ZEA occurred in maize at a rate of 33%, with an average level of 15 μg.kg^{-1} (EFSA 2011), significantly lower than the EU standards. However, investigations in Belgium on thirty samples of maize, wheat, oats, and other cereal-derived foods showed an 80% incidence of ZEA with a mean of 0.106 ± 221 μg.kg^{-1} (De Boevre *et al*., 2012).

A part of the regulated mycotoxins identified in that study, AFG2 and AFB2 aflatoxins, were detected twice with average levels of 1.5 and 18.5 μg.kg^{-1}, respectively, compared with those detected at lower frequencies and concentrations in Iranian wheat samples (0.11–0.34 μg.kg^{-1} and 0.12 μg.kg^{-1}, respectively) (Kardani *et al*., 2023). Because of the high concentrations of AFB2 in durum wheat analyzed, which are beyond the EU limit (4 μg.kg^{-1} in raw cereals and its products derived), it's more prone to aflatoxins contamination. This further exacerbates food safety concerns. Interestingly, T-2 was detected in only one positive sample in our investigations with amounts lower than the LOQ, and this strongly confirms the findings of our assay on the ability of pathogen isolates to produce mycotoxins. It seems plausible that these results are attributed to a combination of factors, like the lack of T-2-producing species, host cultivar type, and harsh local climatic conditions for T-2 biosynthesis.

As was noticed, there is a non-significant correlation between *Fusarium* strains' pathogenicity and mycotoxins' production. Li *et al*. (2023) reported the absence of a link between toxin production and the pathogenicity level of *F. oxysporum* f. sp. *sesami* isolates, as demonstrated by the non-significant correlation (p>0.05). They suggest that the results contribute to a better understanding of host-pathogen interaction and pathogen control mechanisms.

The toxin data reported here revealed co-contamination of Algerian durum wheat with multiple *Aspergillus* and *Fusarium* mycotoxins owing to the occurrence of various toxin-producing species. This exerts a detrimental impact on consumer health, inducing acute and chronic diseases of even more significant concern. No stringent regulation has been stipulated up to now. To the best of our knowledge, investigations on the risk of pathogenic species prevalence and their associated mycotoxins in harvested wheat seeds are currently scarce in Algeria. For

the first time, we indicate the potential mycotoxin profile of *Fusarium* isolates causing Fusarium wilt on wheat, in addition to the natural occurrence and co-occurrence of multi-mycotoxins in durum wheat produced in Algeria. Therefore, this underscores the urgent need to control and regulate mycotoxin levels in cereals as a major challenge that must be taken with more thoughtfulness to safeguard food security.

3.6. Conclusion

This study adds to our understanding of the pathogenicity profile of *Fusarium* strains, including the most aggressive, *F. avenaceum* FusBi7. Furthermore, differences in the behavior of durum wheat types toward pathogenic infections have emerged, highlighting the Cirta variety as the most tolerant to *Fusarium* strain attacks. It should also be highlighted that pathogen isolates recovered from symptomatic ears and kernels can cause wheat crown rot, implying that the two diseases are caused by the same culprit. Additionally, the study revealed that Fusarium wilt induction occurs independently of mycotoxin synthesis.

4. Multiple trophic and climatic factors' impacts on kinetics *Fusarium spp.* growth involved in Fusarium wilt in wheat

4.1. Abstract

Climatic and nutritional factors play an important role in the behavior of pathogenic agents and consequently in the economic losses suffered by cultivated plants. Determining the most suitable conditions for each pathogen is essential for the formulation of phytosanitary management strategies. In this context, studies were carried out in vitro by one-factor-at-a-time method to understand the physiological profile of eighteen *Fusarium* isolates (6 *F. clavum*, 2 *F. avenaceum*, 5 *F. acuminatum*, 2 *F. culmorum*, 1 *F. tricinctum*, 1 *F. microconidium*, and 1 *F. solani)*, identified primarily from Algerian durum wheat**.** Results highlighted a superior discrimination mycelial growth according to the nutritional requirements including culture media, source of carbon and nitrogen as well as the C:N ratio and also according to the levels of pH, degree of salinity, temperatures and relative humidity. The findings of the current study suggested that Czapek Dox Agar medium at 25°C temperature, 95% of relative humidity, pH 7, 2.5 g. L^{-1} of salinity, cellulose as carbon source, peptone as nitrogen source and 10:1 of C:N ratio, all experienced accretion in mycelium growth of *Fusarium* isolates. A great diversity was observed between isolates and species of *Fusarium* studied; different strains of the same species behave distinctly towards the climatic and trophic factors and conversely, different species respond in the same way. These parameters were significant in discriminating isolates into two clusters according to the hierarchical ascending classification.

4.2. Introduction

All living organisms interact actively with their surrounding environments and modulate their physiology to maintain cellular homeostasis (Fangwei *et al*., 2014). Among them, fungal plant pathogens are influenced by environmental factors in growth, survival, dissemination and hence the incidence of fungi and the disease severity (Doohan *et al*., 2003, Yadav *et al*., 2014). The kinetic of fungal phytopathogen growth is also affected by variation in hydrogen, carbon and nitrogen sources (Patel, 2020). Moreover, wide variety of C and N sources could be used by fungi for their ability to release extracellular enzymes that break down complex substrate into readily assimilated compounds (Lee *et al.*, 2007). These fungi do not only decompose plant cell wall polymers to acquire a necessary nutrient source but also digest the cell wall leading to cell penetration and diffusion through plant tissues (An *et al*., 2005).

Besides nutrient limitations, salinity is one of the most stringent abiotic stresses limiting crop growth in agricultural fields (Singh, 2021), and it has been reported to increase the

susceptibility of some crops to soil-borne fungus-like microorganisms (Sanogo, 2004) and fungi (Howell *et al*., 1994). Fungi produce a plethora of biologically active metabolites, e.g., pigments, mycotoxins, phytotoxins and extracellular enzymes, in which their biosynthesis is mostly linked to growth processes and environmental factors from nutrient ratios to light and temperature (Calvo *et al*., 2002; Keller *et al*., 2005). Fungal pigments not only contribute to the survival of the fungal spore by protecting it from environmental stress as UV light but are also an important virulence factor (Calvo *et al*., 2002).

Among the important pathogens of small-grain cereals, *Fusarium* fungi causing Fusarium head blight which seriously impacts the yield and quality of grain by contamination with mycotoxins such as deoxynivalenol and nivalenol (Kawakami *et al*., 2014). FHB results from the development of a complex of at minimal 19 causative agents undergo under *Fusarium* genus, principally by *F. graminearum* and *F. culmorum*. Additionally, other species are minimum repeatedly added agents such as *F. equiseti, F. poae* and *F. cerealis*, and, to a lower range, *F. solani*, *F. verticillioides* and *F. oxysporum* (Bottalico and Perrone, 2002).

Geographical prevalence of the various species is strongly driven by meteorological factors such as temperature and humidity (Bakker *et al*., 2018). Temperatures up to 25°C and high relative humidity enhance both inoculum production and infection by *F. graminearum* (De Wolf *et al.*, 2003). Outbreaks of soil-borne FHB species occurring in seasons with frequent rainfall and high humidity can compromise yield and contaminate wheat and barley grains with dangerous mycotoxins (McMullen *et al*., 2012). The toxins content in grain samples was higher after inoculation at 10°C than after inoculations at 15 or 20°C (Schöneberg *et al*., 2019). However, the interaction of *F. oxysporum* with salt stress varied depending on *formae speciales* and host-plants involved. In fact, increased disease incidence following irrigation with high-salinity water had been reported in several pathosystems including a variety of *F. oxysporum* f. sp. *vasinfectum* (Ragazzi *et al.*, 1994). However, sodium chloride (NaCl) has been used for suppression of Fusarium diseases on many plants such as *F. oxysporum* f. sp. *asparagi* and *F. moniliforme* on asparagus (Elmer, 2003).

The diseases are usually managed through integration of various methods with the aim to suppress the pathogen invasion, multiplication and survival. Plants and pathogens coevolved in nature. Plant growth conditions may be altered to create the worst conditions for the pathogen development but without sacrificing the yield. The environmental factors have a significant impact on the expression of the virulence genes and the pathogenic behavior of soil-borne phytopathogens (An *et al*., 2020). Herein, the present work depicts

the role of different climatic and trophic factors to understand ecological survival of etiological agents and their metabolome expression which will be helpful for effective *Fusarium* disease management strategy in the field.

4.3. Material and methods

4.3.1. Fungal material

Eighteen *Fusarium* isolates isolated from diseased durum wheat seeds and ears and successfully identified in previous chapter 1, were used in various experimentation to determine their environmental and nutritional properties. All isolates were maintained on potato sucrose agar (PSA) at 4°C and are codified as it is: *F. clavum* (FusBi8, FusBi1, FusBo25, FusBo28, FusBo49, FusBi2), *F. culmorum* (FusBo50, FusBo59), *F. microconidium* (FusBo26), *F. avenaceum* (FusBi7, FusBi21), *F. tricinctum* (FusBi6), *F. acuminatum* (FusBi23, FusBo33, FusBi15, FusBo11.5, FusBo6.12), and *F. solani* (FusBo35).

4.3.2. Effect of various trophic factors on mycelial growth of *Fusarium* isolates

4.3.2.1. Culture media

Four different culture media, namely potato sucrose agar (PSA) (Samson *et al*., 2002), Czapek Dox Agar (CDA) (Jo *et al*., 2010), Spezieller Nährstoffarmer Agar (SNA) (Leslie and Summerell, 2008) and Wheat Grain Extract Agar (WGEA) (Maurya *et al*., 2019) were screened to determine the optimal medium for the mycelial growth of eighteen isolates. Streptomycin sulfate (0.5 g. L^{-1}) was added to avoid any bacterial contamination. Media were pour-plated and inoculated centrally with mycelia discs (5 mm diameter) from seven days old culture and incubated at 25°C in the dark for 7 days.

4.3.2.2. Carbon sources

The nutritional requirements for optimal mycelial growth of the *Fusarium* isolates were assessed on the solid basal medium CDA supplied with various nutrient sources such as carbon and nitrogen compounds. Streptomycin sulfate (0.5 g. L^{-1}) was also added to avoid any bacterial contamination. The optimum pH of the medium and the required temperature conditions were applied.

In order to determine the most suitable carbon sources for mycelial growth, a modified method of Jo *et al*. (2010) was used, where various carbon sources including glucose, sucrose, and cellulose were added to the basal medium at a concentration of 3% (w/v). Media were dispensed into flasks, and then sterilized at 121°C for 20 min. After cooling, a

20 mL sterilized medium was poured into 8.5 cm sterile Petri dishes. The Petri dishes containing solidified medium were centrally inoculated with 5 mm diameter mycelial disc from actively growing cultures, and incubated under the required culture conditions.

4.3.2.3. Nitrogen sources

To investigate the required nitrogen sources for the mycelial growth, a modified method of Jo *et al*. (2010) was used, where the basal medium was supplemented with one-fifth of each nitrogen sources, such as valine, leucine, arginine, asparagine and peptone at a concentration of 0.3% (w/v). Media were dispensed into flasks, and then sterilized at 121 °C for 20 min. Culture plates were prepared, inoculated and incubated in the same manner as described in the previous experiment.

4.3.2.4. Carbon to nitrogen (C:N) ratio

For the optimization of the C:N ratio, the most favorable carbon and nitrogen sources form the last two experiments, i.e., sucrose and peptone were selected. The preparation of the different C:N ratio (1:1, 10:1, and 30:1) was made following the method of Jo *et al*. (2010) using the basal medium CDA. Culture plates were prepared, inoculated and incubated as described in the preceding section.

4.3.3. Effect of various climatic factors on mycelial growth of *Fusarium* isolates

The best medium from the previous experiment was used to evaluate the radial mycelial growth of the causal pathogen at various temperature, pH, salinity and relative humidity. In sterilized petri dishes, 20 mL of the sterilized media was poured. Inoculations were made with 5 mm diameter fungal plug from actively growing fungal cultures and were incubated at 25°C in the dark for 7 days.

4.3.3.1. Temperature regimes

The effect of temperature on mycelial growth was studied by growing the fungal cultures on the selected medium at five different temperature regimes (4, 22, 25, 28 and 37°C).

4.3.3.2. Relative humidity

Fusarium species were optimized for their relative humidity (RH). Thus, five different levels of RH (50, 74, 80, 95 and 100%) of the selected medium were maintained according to Benaouali (2015) by taking accurate weight of NaCl, and then dissolved in 100 mL of deionized distilled water to obtain the required levels of RH. The plates were converted and 9 mL of each prepared solution was poured into the cover of each Petri dish.

4.3.3.3. pH levels

The influence of pH on mycelial growth was studied by growing the fungal cultures on the selected medium that was adjusted to different pH levels (4.5, 7.0 and 8.5) using 0.5 N sodium hydroxide (NaOH) or 10% acetic acid (CH_3COOH) accordingly.

4.3.3.4. Salinity

To evaluate salinity tolerance, 5 mm actively growing mycelia discs of different *Fusarium* spp. isolates were cultured on the selected medium plates amended with NaCl at 2.5 g. L^{-1}, 5 g. L^{-1} and 10 g. L^{-1}concentrations.

4.3.4. Measurement of mycelial growth and data analysis

In this investigation, all experiments were done in triplicate and the mycelial growth diameter is recorded on the 3rd, 5th and 7th day after inoculation. Statistical analyzes were carried out for all the parameters measured where the culture media, carbon, nitrogen and their C:N ratio, temperature, relative humidity, pH and salinity were the fixed factors and the averages were separated by *Fusarium* isolates and incubation time at $p \leq 0.05$ using Excel 2010 and GraphPad Prism9 software.

4.4. Results

4.4.1. Effect of various trophic factors on mycelial growth of *Fusarium* isolates

4.4.1.1. Culture media

The effect of several nutrient media, including PSA, CDA, SNA and WGEA on the *Fusarium* spp. growth was studied. All the strains of *Fusarium* studied grew in the four culture media and the evolution of mycelial growth per day is almost similar, showing no significant difference at the 5% threshold. However, *Fusarium* strains show a noticeable difference and preference for certain culture media over others. Thus, Czapek Dox Agar medium is the most favorable medium since the mycelial growth average of *Fusarium* strains was the greatest on the 3rd, 5th and 7th day of incubation equal to 36.48 ± 1.65, 62.48 ± 2.77 and 74.94 ± 2.54 mm, respectively (Figure 4.1). It emerges that 13 strains out of the 18 studied recorded 85 mm, i.e., the maximum possible (Figure 4.2). Conversely, the WGEA proved to be the least favorable medium for mycelial growth of *Fusarium* strains, with very low averages equal to 28.74 ± 1.68, 44.19 ± 3.00 and 55.74 ± 3.63 mm, on the 3rd, 5th and 7th day of incubation, respectively. This represents only half of the mycelial growth obtained with the CDA medium (Figure 4.1). At the species level, on the 7th day of incubation, three strains (FusBi1, FusBi21, FusBo49) showed a highest mycelial growth

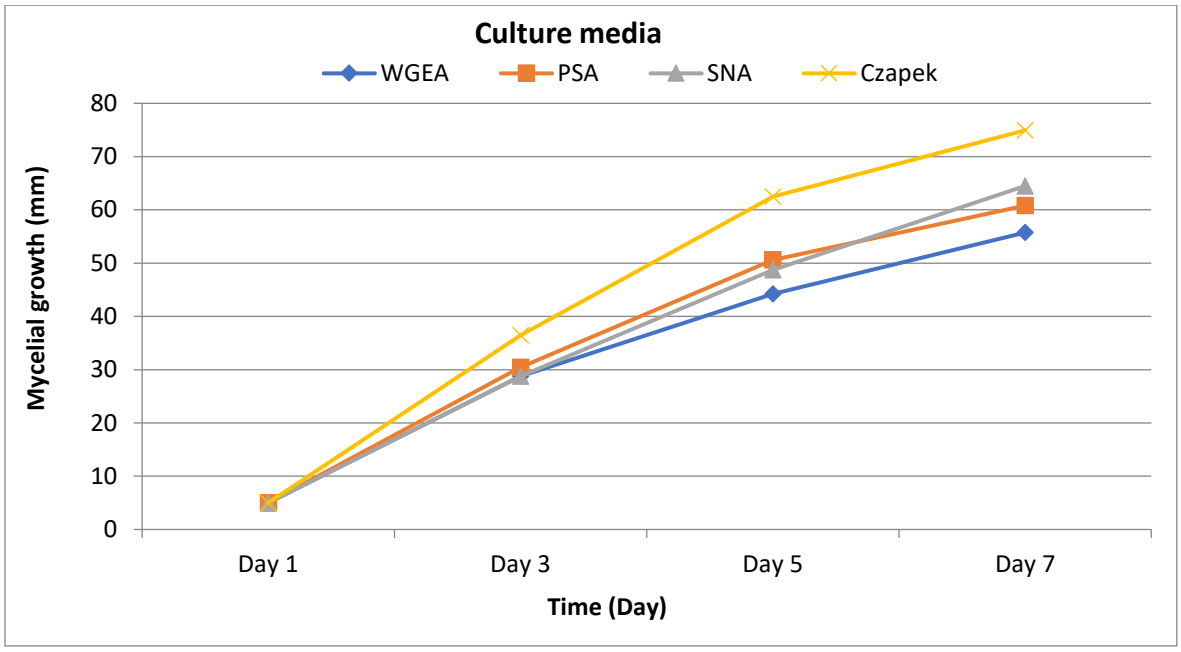


Figure 4.1. Effect of culture media on the growth kinetics of *Fusarium* isolates (n = 54).

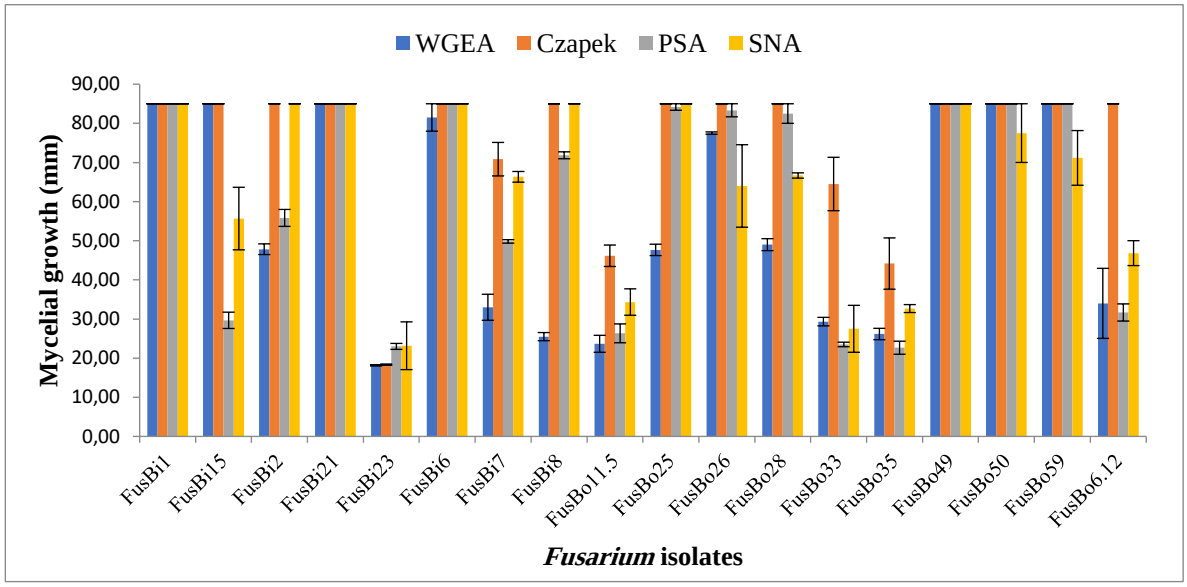


Figure 4.2. Effect of culture media on mycelial growth of *Fusarium* isolates on day 7 of incubation. Data are mean ± SEM (n = 3), error bars represent standard errors.

and reached the edges of the Petri dish, i.e., 85 ± 0.0 mm in diameter, whatever the medium. Nevertheless, three strains (FusBi23, FusBo11.5 and FusBo35) were characterized by a very weak mycelial growth in all culture media (Figure 4.2). According to the results obtained, we note the great variability between *Fusarium* strains; thus, those belonging to the same species (*F. acuminatum*: FusBi15, FusBi23, FusBo11.5, FusBo6.12 and FusBo33) gave quite different growth levels. However, strains which belong to

different *Fusarium* species including *F. clavum* (FusBi1), *F. avenaceum* (FusBi21), and *F. culmorum* (FusBo50), have given levels of mycelial growth quite close in the all medium (Figure 4.2).

4.4.1.2. Carbon sources

All the carbon sources were suitable for the fungus growth (Figure 4.3). At three days of incubation, the *Fusarium* strains use more cellulose as a Carbon source, resulting in mycelial growth equal to 19.09 ± 0.75 mm, slightly better than 17.29 ± 0.87 and 18.98 ± 0.82 mm recorded with glucose and sucrose, respectively (Figure 4.3).

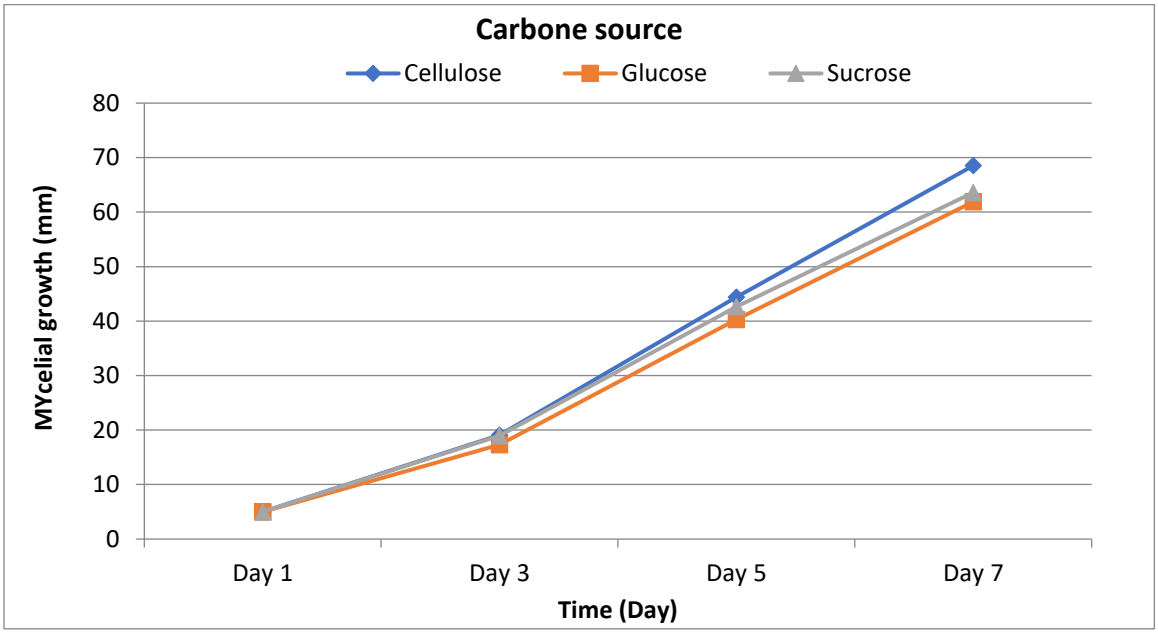


Figure 4.3. Effect of carbon sources on the growth kinetics of *Fusarium* isolates (n = 54).

This preference to cellulose still continues when the colonies of *Fusarium* are well established on the 5th and 7th day of incubation, with average diameter growth equal to the 44.37 ± 1.33 and 68.52 ± 1.70 mm respectively. All *Fusarium* strains registered at the 7th day of incubation an average diameter growth equal to the 68.52 ± 1.70, 63.59 ± 2.90 and 61.89 ± 2.71 mm, for cellulose, sucrose and glucose, respectively. Individually, ANOVA analysis shows that the mycelial growth of *Fusarium* isolates regarding the source of carbon was significantly different at $P<0.05$. The results reveal that FusBo59 has an excellent growth with the three sources of carbon equal to 84.33 ± 0.67, 85.00 ± 0.00 and 84.44 ± 1.00 mm at the 7th day of incubation with cellulose, glucose and sucrose, respectively, while the FusBi23 showed poorer growth than the others on all carbon sources tested, resulting to 50.33 ± 1.45, 19.67 ± 2.33 and 17.67 ± 2.60 mm, to the same carbon source, respectively (Figure 4.4).

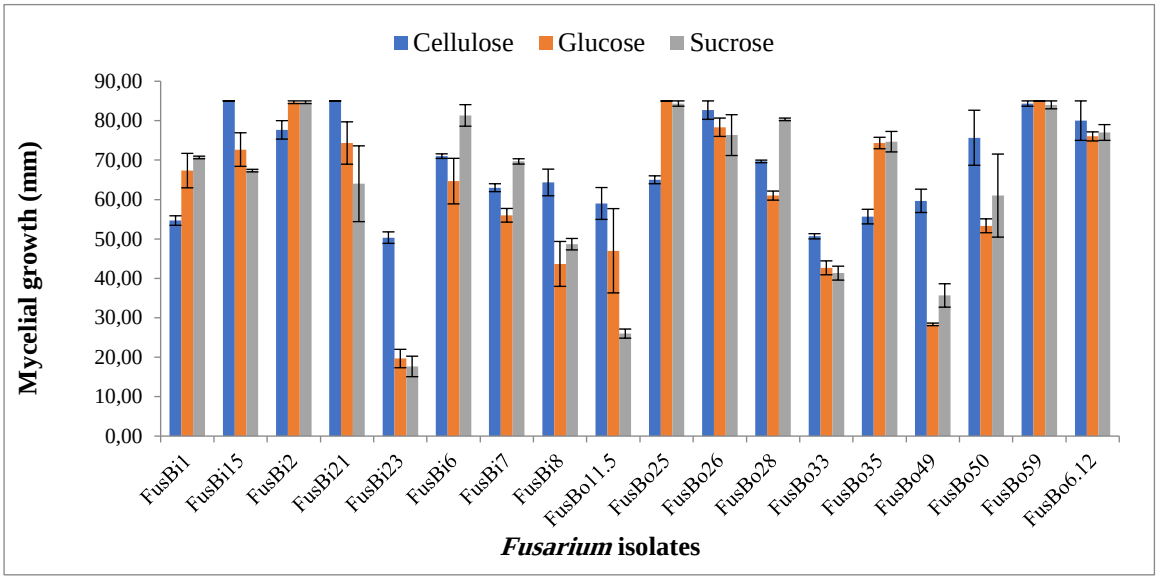


Figure 4.4. Effect of carbon sources on mycelial growth of *Fusarium* isolates on day 7 of incubation. Data are mean ± SEM (n = 3), error bars represent standard errors.

From the practical point of view, sucrose is an excellent alternative for the carbon source due to its simplicity of use and affordability as compared to other carbon sources. Thus, sucrose was chosen as the carbon source in the subsequent tests.

4.4.1.3. Nitrogen sources

Based on a visual evaluation, cultures grown on peptone offered the maximum pigment compared to other sources (Figure 4.5). As a result, the degree of pigment intensification could be correlated with the mycelial growth which is in turn dependent on the nitrogen source. Previous studies revealed that pigments production by fungi is influenced by several factors including incubation time, pH of the culture medium, carbon sources, nitrogen sources, incubation temperature, inoculum density and carbon source concentration (Agboyibor *et al*., 2019; Elattaapy and Selim, 2020; Deshaware *et al*., 2021). In the present study, the variation in the nitrogen source affected not only the mycelial growth, but also the pigmentation.

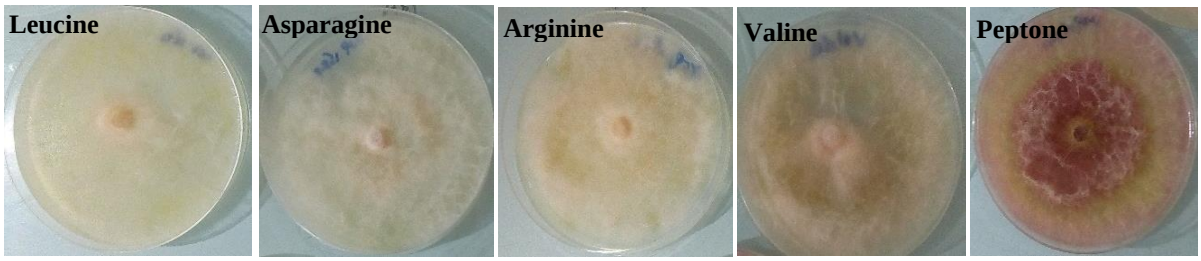


Figure 4.5. Effect of different nitrogen sources on pigments production observed in *F. acuminatum* (FusBi15 isolate) colonies.

According the five organic nitrogen sources examined (arginine, asparagine, valine, leucine and peptone), the mycelial growth of the 18 isolates of *Fusarium* is progressive according to the incubation time (Figure 4.6). Peptone was found to be the best source of nitrogen for all the isolates of *Fusarium*, with average equal to 38.76 ± 1.63, 66.98 ± 2.59 and 75.81 ± 2.35 mm after 3rd, 5th and 7th day of incubation, respectively. The lowest fungal growth was recorded on media supplied with arginine (Figure 4.6).

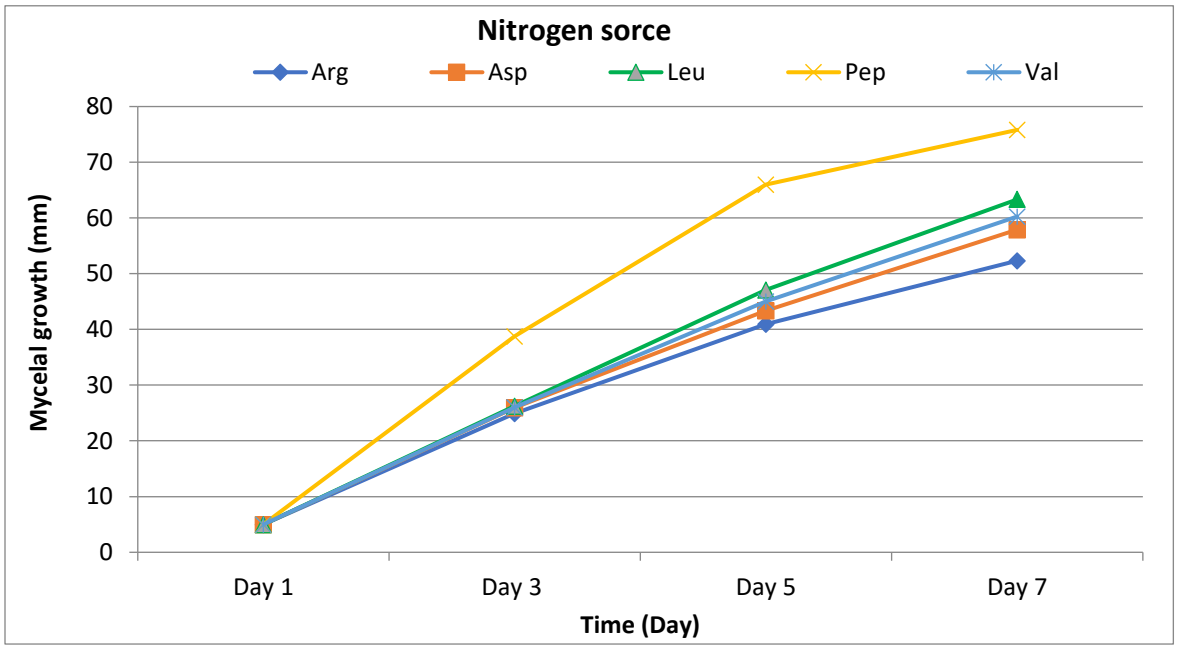


Figure 4.6. Effect of nitrogen sources on the growth kinetics of *Fusarium* isolates (n = 54).

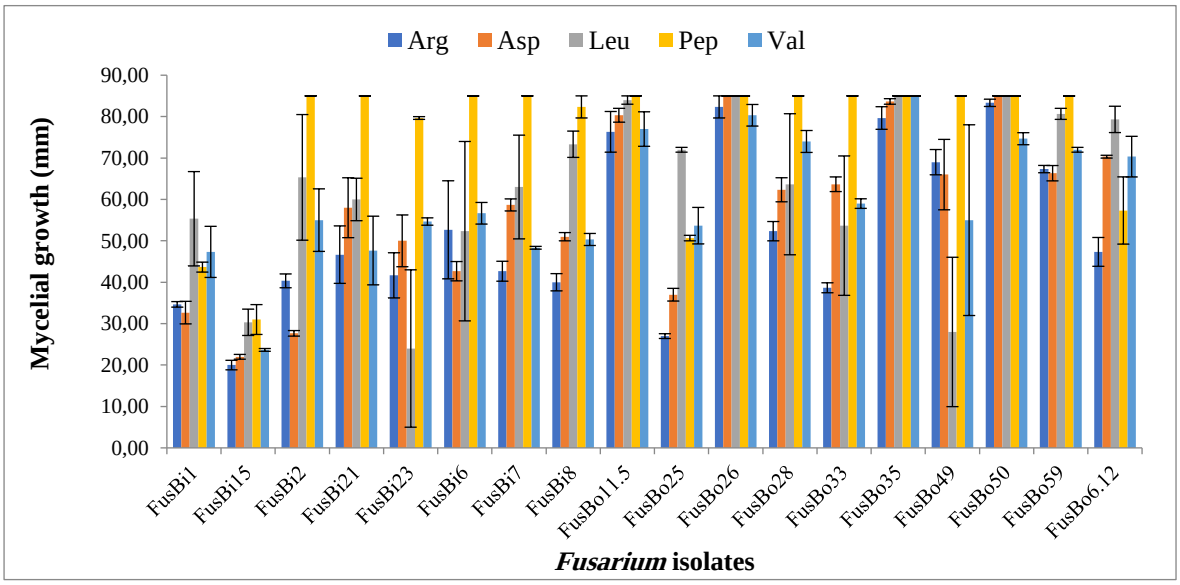


Figure 4.7. Effect of nitrogen sources on mycelial growth of *Fusarium* isolates on day 7 of incubation. Data are mean ± SEM (n = 3), error bars represent standard errors.

At the species level, the FusBo50 registered the highest mycelia growth equal to for 83.67 ± 0.88, 85.00 ± 0.00, 85.00 ± 0.00, 85.00 ± 0.00 and 79.67 ± 1.45 mm at the 7th day of incubation for arginine, asparagine, leucine, peptone, and valine, respectively, while FusBi15 is characterized by slower growth than the others on all nitrogen sources used with only 20.00 ± 1.15, 22.00 ± 0.58, 30.33 ± 3.18, 31.00 ± 3.61 and 23.67 ± 0.33 mm to the same nitrogen source, respectively (Figure 4.7).

4.4.1.4. Carbon to nitrogen (C:N) ratio

Statistical analysis revealed a highly significant difference between C:N ratios and incubation time of *Fusarium* strains. Thus, the amount of carbon supplementing the culture medium compared to the amount of nitrogen can enable mycelial growth by going from an equivalent ratio 1:1 giving an average growth equal to 11.53 ± 0.76, 27.12 ± 1.93, 44.43 ± 3.15 mm to a 10:1 ratio, producing 38.19 ± 1.53, 65.31 ± 2.53, 75.81 ± 2.35 mm, recorded after 3, 5 and 7 days of incubation, respectively. On the other hand, the 30:1 ratio inhibited the mycelial growth giving only 14.73 ± 1.11, 34.25 ± 2.93 and 51.03 ± 3.59 mm after 3, 5 and 7 days of incubation (Figure 4.8). Individually, FusBo6.12 showed the highest mycelial growth with all three ratios, recording 81.00 ± 1.00, 85.00 ± 0.00, 85.00 ± 0.00 mm at the 7th day of incubation for 1:1, 10:1, and 30:1 ratio, respectively; while the *Fusarium* strain FusBi21 proved to be the least beneficial, recording only 16.17 ± 0.88, 31.00 ± 3.61 and 25.00 ± 2.02 mm at the 7th day of incubation for 1:1, 10:1, and 30:1 ratio, respectively (Figure 4.9).

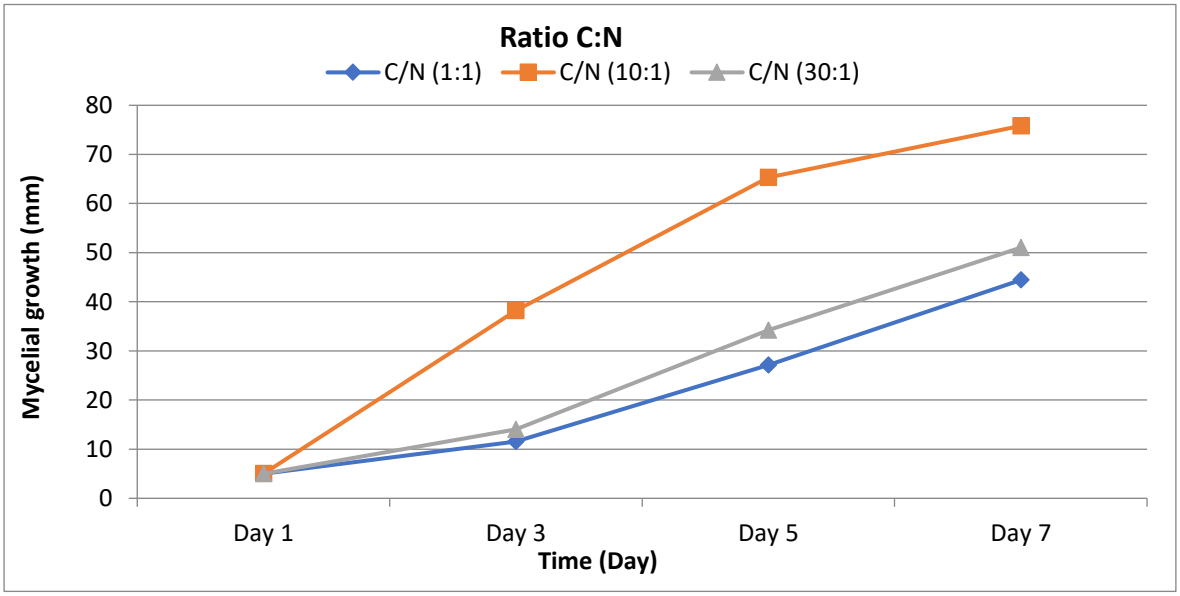


Figure 4.8. Effect of carbon to nitrogen (C:N) ratio on the growth kinetics of *Fusarium* isolates (n = 54).

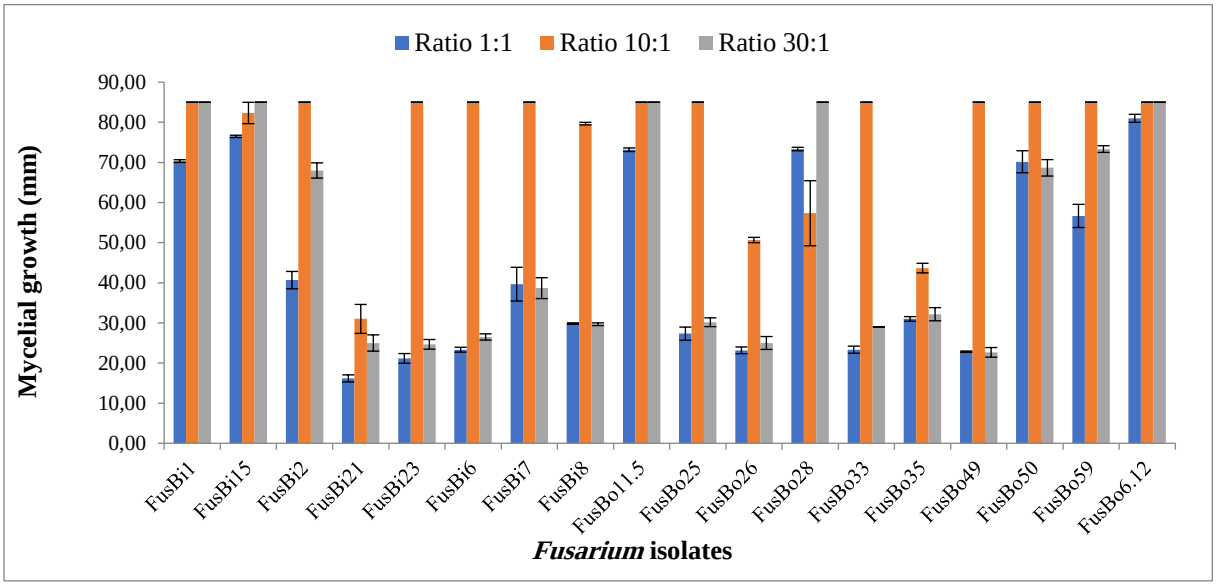


Figure 4.9. Effect of carbon to nitrogen (C:N) ratio on mycelial growth of *Fusarium* isolates on day 7 of incubation. Data are mean ± SEM (n = 3), error bars represent standard errors.

4.4.2. Effect of various climatic factors on mycelial growth of *Fusarium* isolates

4.4.2.1. Temperature regimes

The results of the effect of temperature on the mycelial growth of 18 *Fusarium* strains confirm the data already known for the most favorable conditions for fungi in general. Thus, it appears that the temperatures of 4 and 37°C largely inhibited mycelial growth giving only 7.3 ± 0.26, 11.63 ± 0.64, 16.78 ± 1.18, and also 5.56 ± 0.15, 6.01 ± 0.25, 6.49 ± 0.35 mm after 3, 5 and 7 days of incubation, respectively (Figure 4.10). In contrast, very little difference in the results of mycelial growth obtained with temperatures of 22, 25 and 28°C after the same incubation period mentioned above. However, a great stimulation of the 18 *Fusarium* strains seems to be visible with the results obtained with the temperature of 25°C (Figure 4.10). Individually, at the species level, FusBo50 and FusBo59 strains recorded the highest mycelium growth reaching the maximum radial diameter equal to 85 ± 0.00 mm with the most favorable temperatures and seems not to suffer too much from the low temperature of 4°C giving a diameter of 39.67 ± 1.86 mm, while FusBi23 exhibited slower growth (6.83 ± 0.44, 18.83 ± 2.89, 23.00 ± 0.76, 18.00 ± 0.50, 5.00 ± 0.00 mm) than the others on all temperature regimes (4, 22, 25, 28 and 37°C) which leads us to presume that it is an intrinsic character of the strain (Figure 4.11).

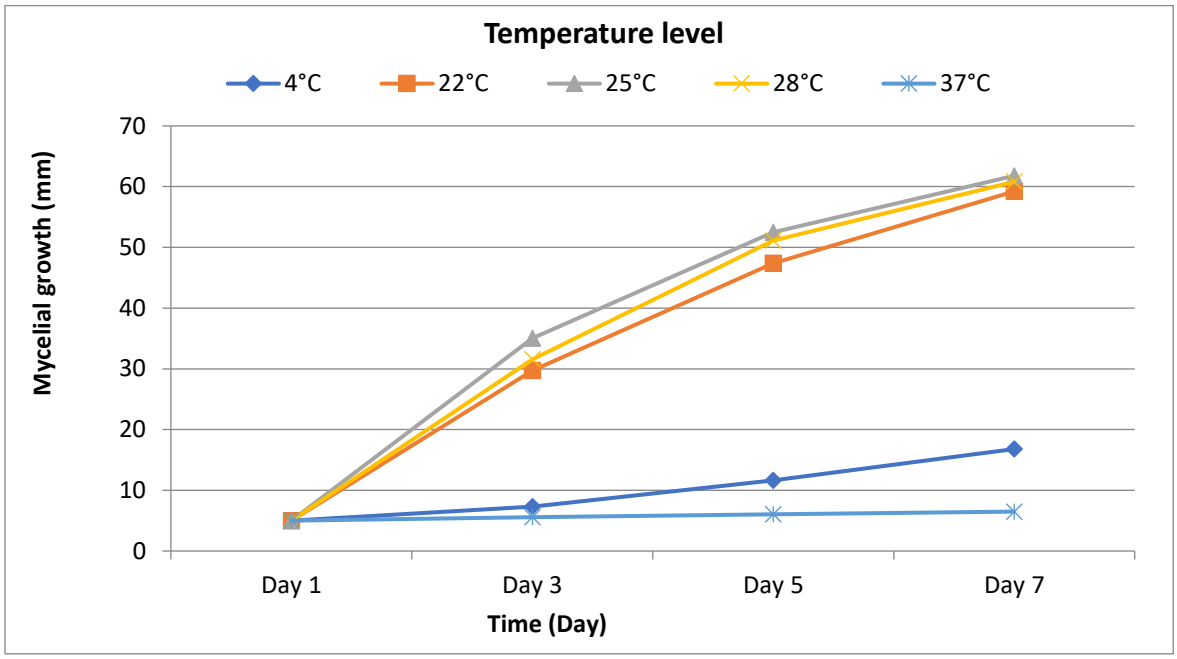


Figure 4.10. Effect of temperature on the growth kinetics of *Fusarium* isolates (n = 54).

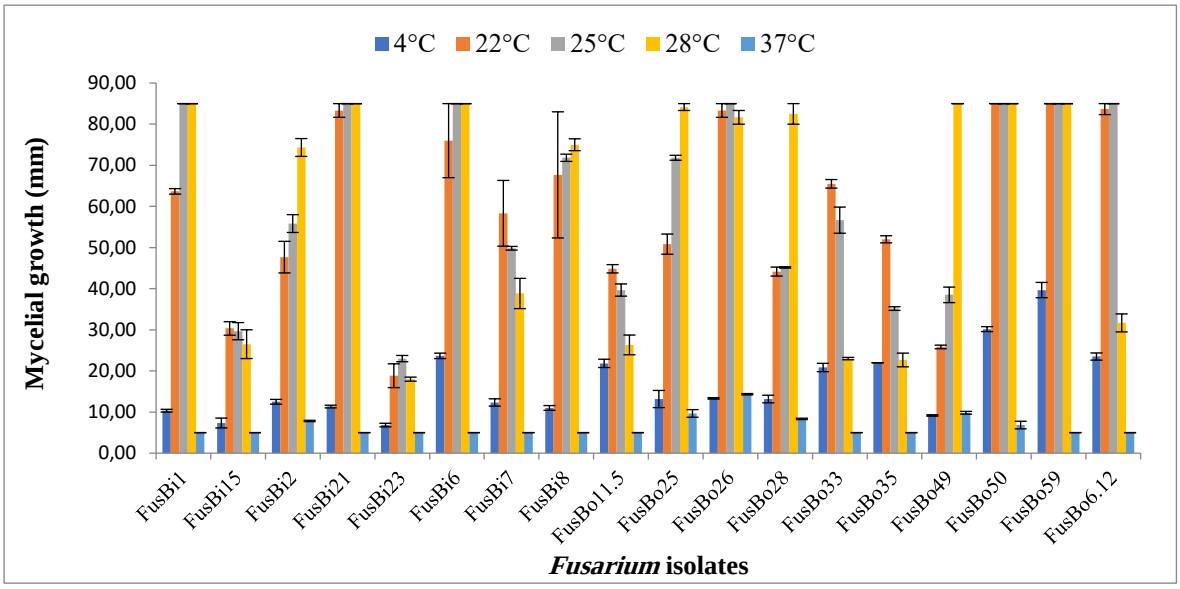


Figure 4.11. Effect of temperature on mycelial growth of *Fusarium* isolates on day 7 of incubation. Data are mean ± SEM (n = 3), error bars represent standard errors.

4.4.2.2. Relative humidity

It is obvious from results (Figures 4.12, 4.13, and 4.14) that the mycelial growth of 18 isolates of *Fusarium* species tested demonstrated to be significantly affected by the different relative humidity levels. The maximum growth was found at 95%, relative humidity, with average diameter of 58.00 ± 3.77 mm, after 7 days of incubation. The lowest growth was noted in all the isolates evaluated at the relative humidity (RH) levels of 50% and at 75%, with average diameter of 17.89 ± 1.32 and 23.06 ± 1.90 mm, respectively (Figure 4.12). On an individual level, the FusBi6 strain seems to be the most adapted to the different relative humidity levels (50%, 75%, 80%, 95%, and 100%) with average mycelial growth equal to 38.50 ± 3.12, 55.00 ± 1.17, 85.00 ± 0.00, 85.00 ± 0.00, and 85.00 ± 0.00 mm after 7 days of incubation, respectively. Moreover, in addition to the FusBi23 strain which stood out for its low growth regardless of the factor studied, the FusBi8 strain seems to be affected by humidity with fairly low growth levels equal to 11.50 ± 7.47, 7.33 ± 3.92, 16.00 ± 2.32, 19.33 ± 0.00 and, 19.17 ± 0.00 mm after 7 days of incubation, respectively, under the same humidity conditions as previously mentioned (Figure 4.13).

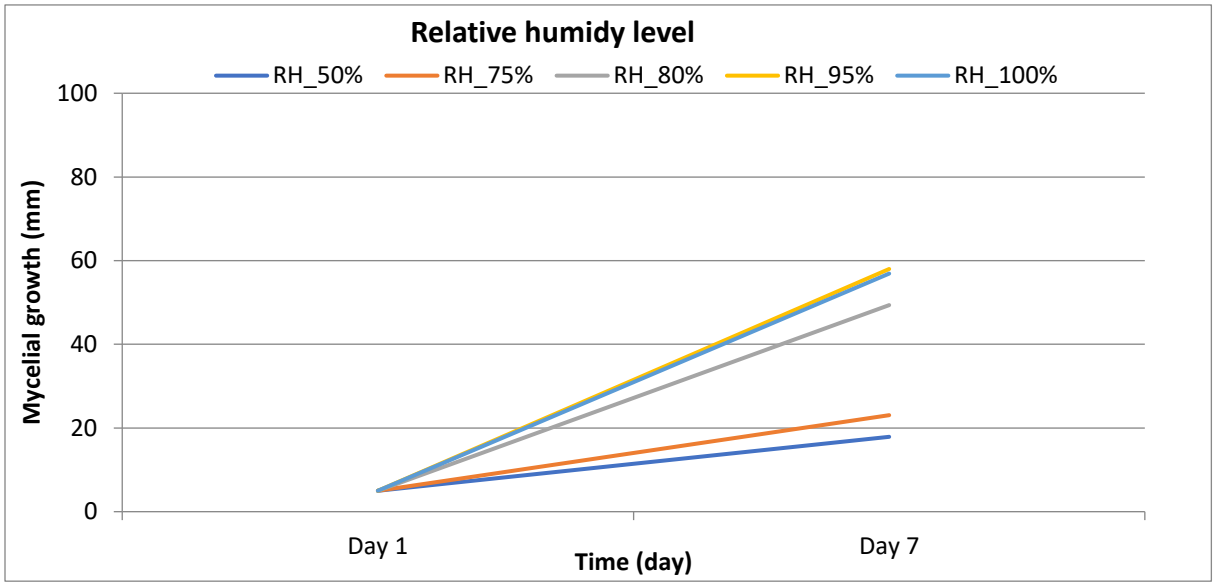


Figure 4.12. Effect of relative humidity (RH) on the growth kinetics of *Fusarium* isolates (n = 54).

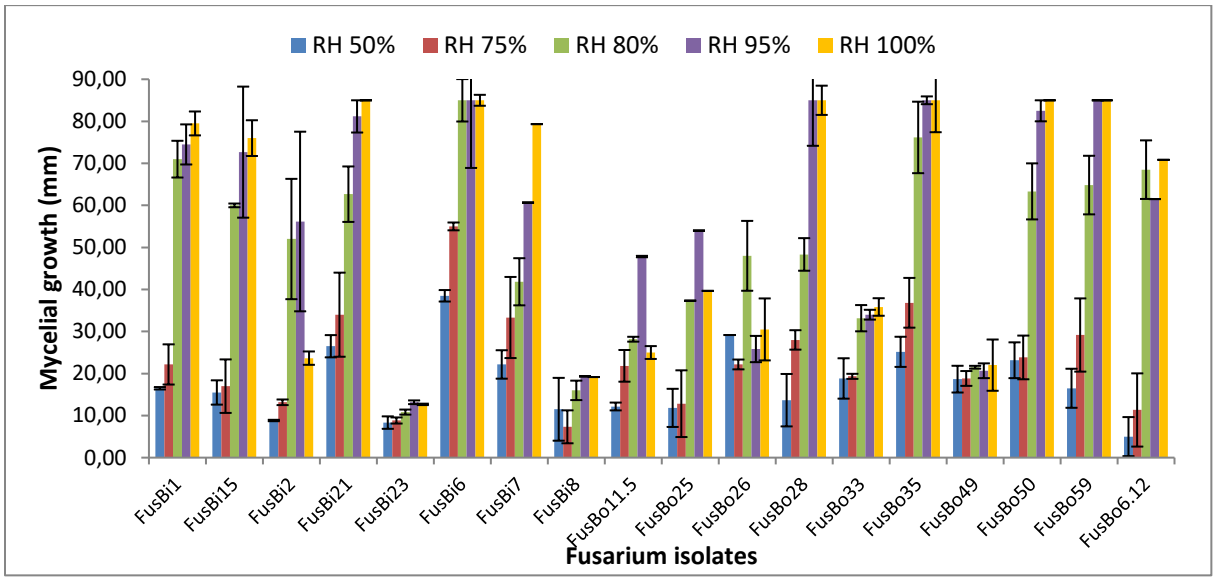


Figure 4.13. Effect of relative humidity (RH) on mycelial growth of *Fusarium* isolates on day 7 of incubation. Data are mean ± SEM (n = 3), error bars represent standard errors.

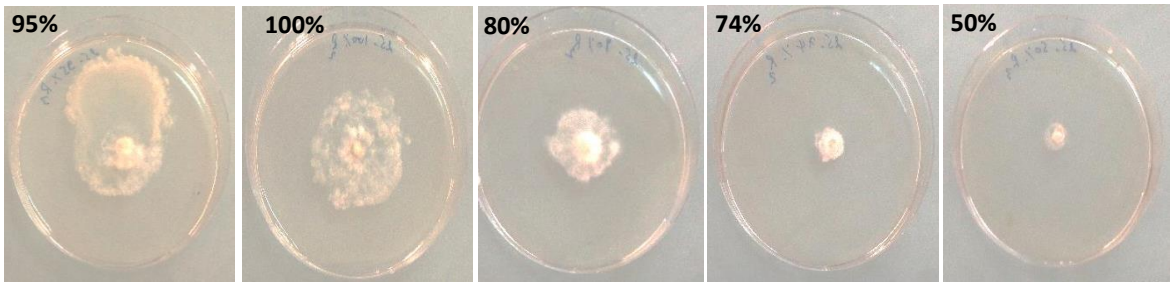


Figure 4.14. Effect of different relative humidity (RH) levels on mycelial growth of *F. clavum*, (FusBo25 isolate).

4.4.2.3. pH levels

The mycelial growth and pigment production were shown to be significantly impacted by the pH of the medium. All *Fusarium* isolates grew well at all pH levels and growth was gradual with incubation time. The highest growth of *Fusarium* isolates was observed at pH 7 was by giving 36.44 ± 1.25, 60.98 ± 2.28 and 73.69 ± 1.94 mm after 3, 5 and 7 days of incubation respectively. These data were very similar with pH 8.5, but were slightly higher than those found with pH 4.5 where we recorded 27.89 ± 1.11, 51.48 ± 2.58 and 63.52 ± 2.70 mm under the same culture conditions, respectively (Figure 4.15). The highest mycelial growth was noted with 5 strains (FusBi15, FusBi21, FusBo28, FusBo50 and FusBo59) reaching the maximum radial diameter equal to 85 ± 0.00 mm after 7 days of incubation with the different pH levels. The rule of the FusBi23 strain with the lowest mycelial growth is confirmed in the case of the impact of pH on mycelial growth. It is

followed by the FusBo49 strain which seems to be affected by the variation of the hydrogen potential with rather low growth rates equal to 36.67 ± 1.45, 48.00 ± 1.53 and 48.33 ± 1.20 mm, after 7 days of incubation, with pH 4.5, 7 and 8.5 respectively (Figure 4.16).

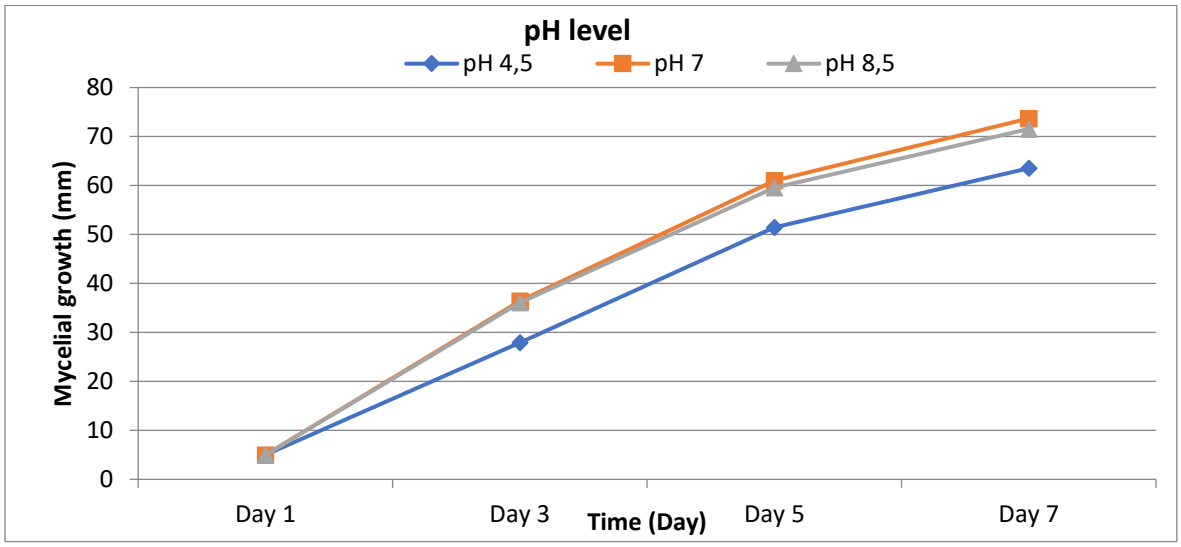


Figure 4.15. Effect of pH on the growth kinetics of *Fusarium* isolates (n = 54).

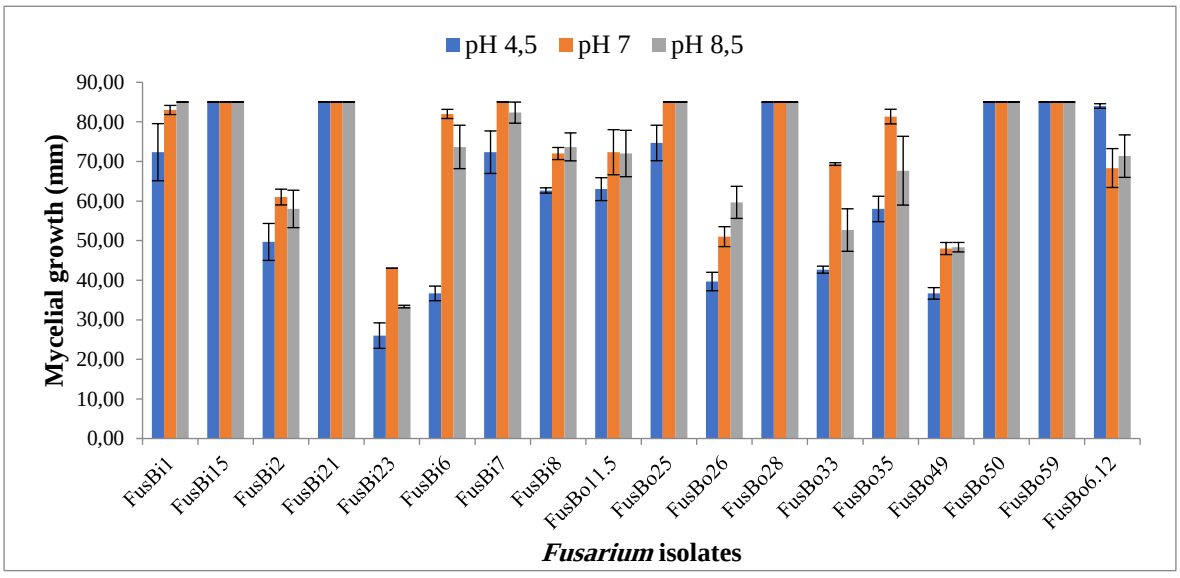


Figure 4.16. Effect of pH on mycelial growth of *Fusarium* isolates on day 7 of incubation. Data are mean ± SEM (n = 3), error bars represent standard errors.

4.4.2.4. Salinity

The effect of salinity concentration was studied through the supplementation of the medium culture by three levels of NaCl (2.5, 5 and 10 g. L^{-1}). Based on the results, the mycelial growth of the *Fusarium* isolates evolved positively with incubation time and the statistical analysis gave a non-significant effect for NaCl concentrations and time of incubation. Slight difference in mycelium growth was noticed between NaCl concentration by at the 3rd day of incubation (24.81 ± 0.94, 23.43 ± 0.95, 23.09 ± 0.98) and the average at the 7th day equal to 74.36 ± 2.45, 71.49 ± 2.68 and 72.56 ± 2.3, for concentrations of 2.5, 5 and 10 g. L^{-1}, respectively (Figure 4.17). Through the above screening protocol, 10 out of 18 strains tested (FusBi1, FusBi15, FusBi2, FusBi21, FusBo28, FusBo35, FusBo50, FusBo59, FusBi7 and FusBo6.12) were detected as salt-tolerant up to 10 g. L^{-1} of salt concentration. The only strain that really stands out from the rest is FusBi6 which only gave radial diameters equal to 27.67 ± 1.36, 25.83 ± 0.73 and 29.83 ± 2.77 mm after 7 days of incubation in culture media with concentrations of around 2.5, 5 and 10 g. L^{-1}, respectively (Figure 4.18).

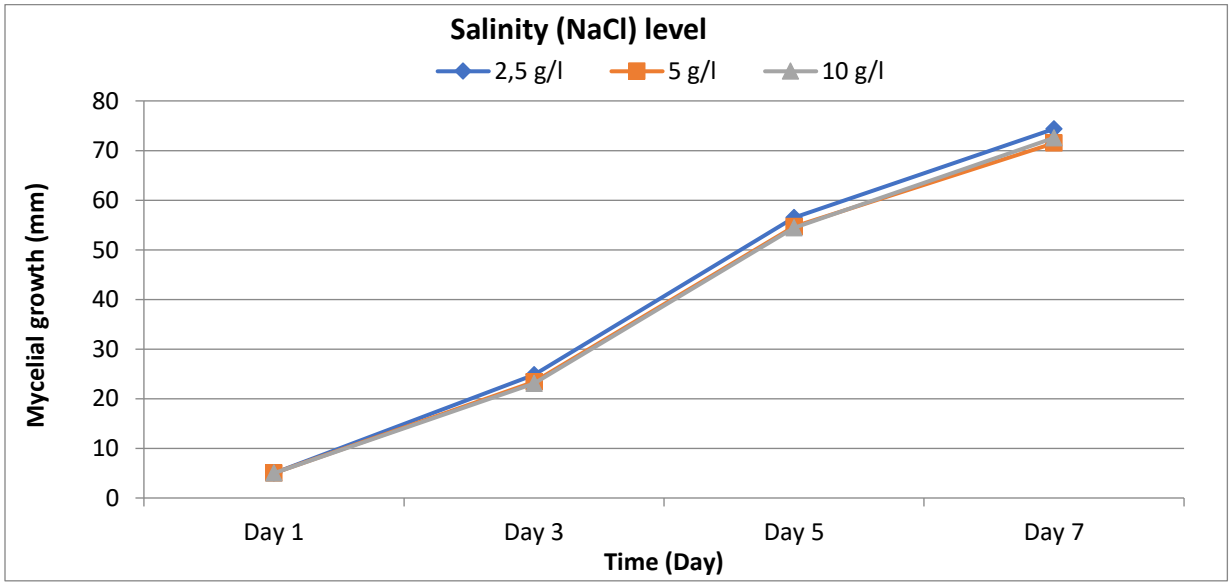


Figure 4.17. Effect of salinity on the growth kinetics of *Fusarium* isolates (n = 54).

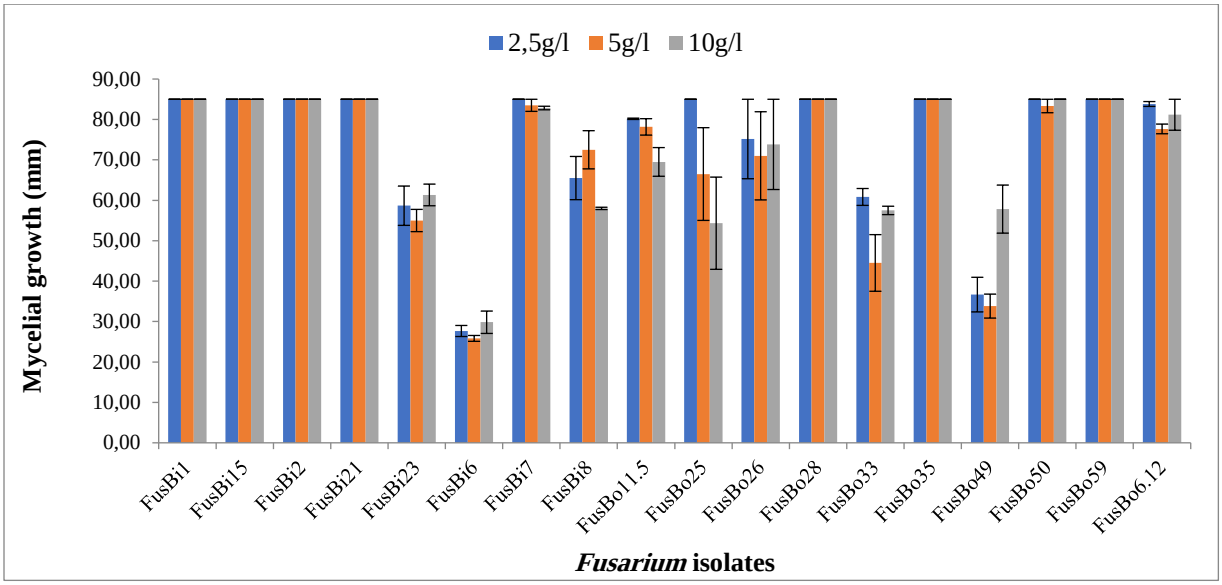


Figure 4.18. Effect of salinity on mycelial growth of *Fusarium* isolates on day 7 of incubation. Data are mean ± SEM (n = 3), error bars represent standard errors.

4.4.3. Ascending hierarchical classification of *Fusarium* isolates

All physicochemical and physiological test results were compiled in a summary matrix and an ascending hierarchical classification was established. The purpose of which is to aggregate *Fusarium* strains into homogeneous clusters. Thus, the optimal classification retained gives us two clusters where the decomposition of the inertia was equal to 76.91% in intra-class and 23.09% in inter-class. These results confirm the great variability between the two clusters and the great homogeneity of each cluster. The first cluster can in turn be subdivided into two sub-clusters. The sub-cluster-a includes the strains: FusBi1, FusBi15, FusBi21, FusBi7, FusBo28, FusBo50, FusBo59 and FusBo6.12, while the sub-clusters-b includes FusBi2, FusBi8, FusBo25, FusBo26, FusBo49 and FusBi6. However, the second cluster includes FusBi23, FusBo11.5 and FusBo33, in addition to FusBo35 which differs from the rest of the group (Figure 4.19). The two clusters are distinct in mycelial growth in terms of optimum temperature requirements in culture media and are slightly similar with respect to pH and relative humidity (Figure 4.20).

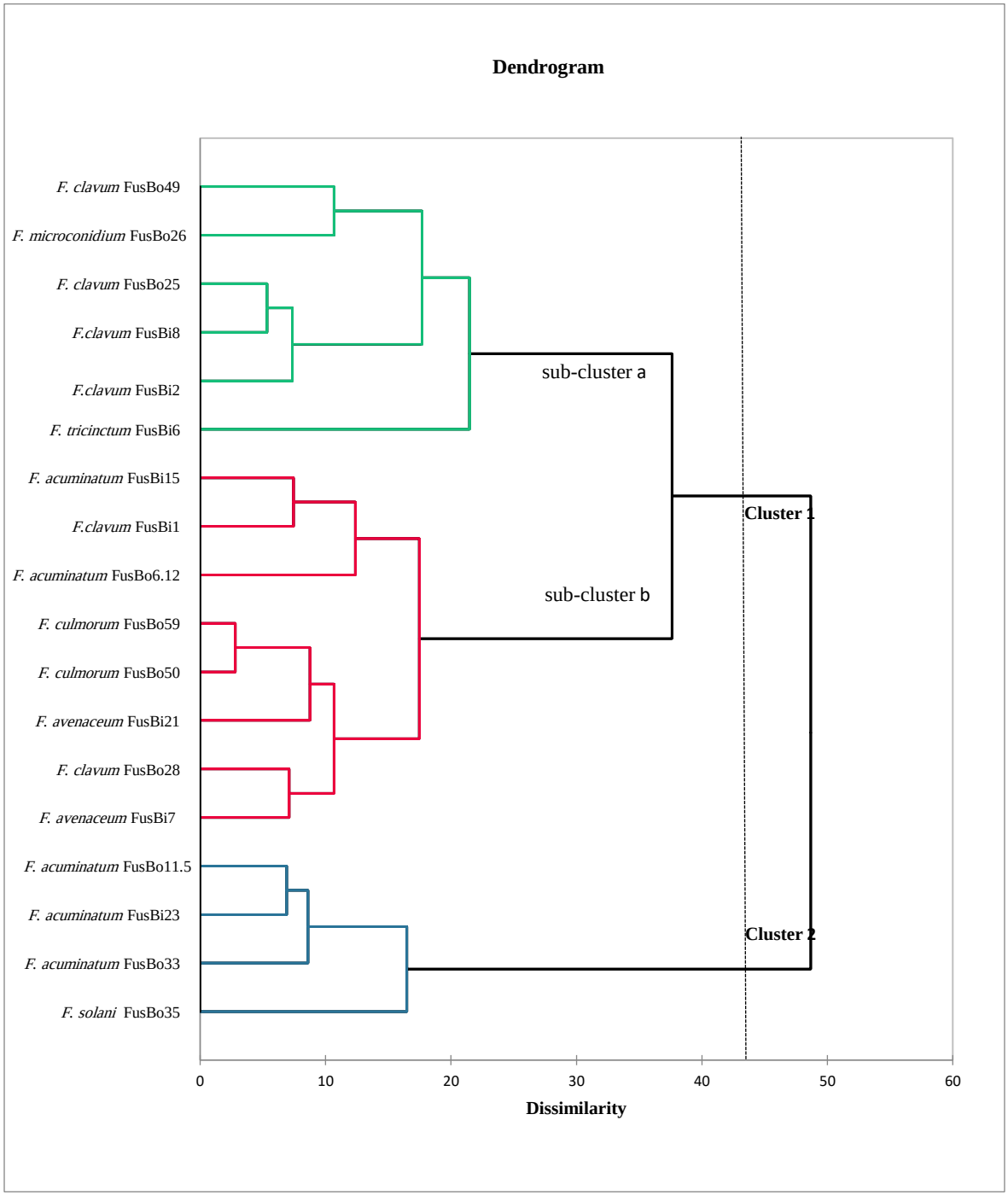


Figure 4.19. Hierarchical ascending classification of *Fusarium* strains according to the climatic and trophic parameters studied.

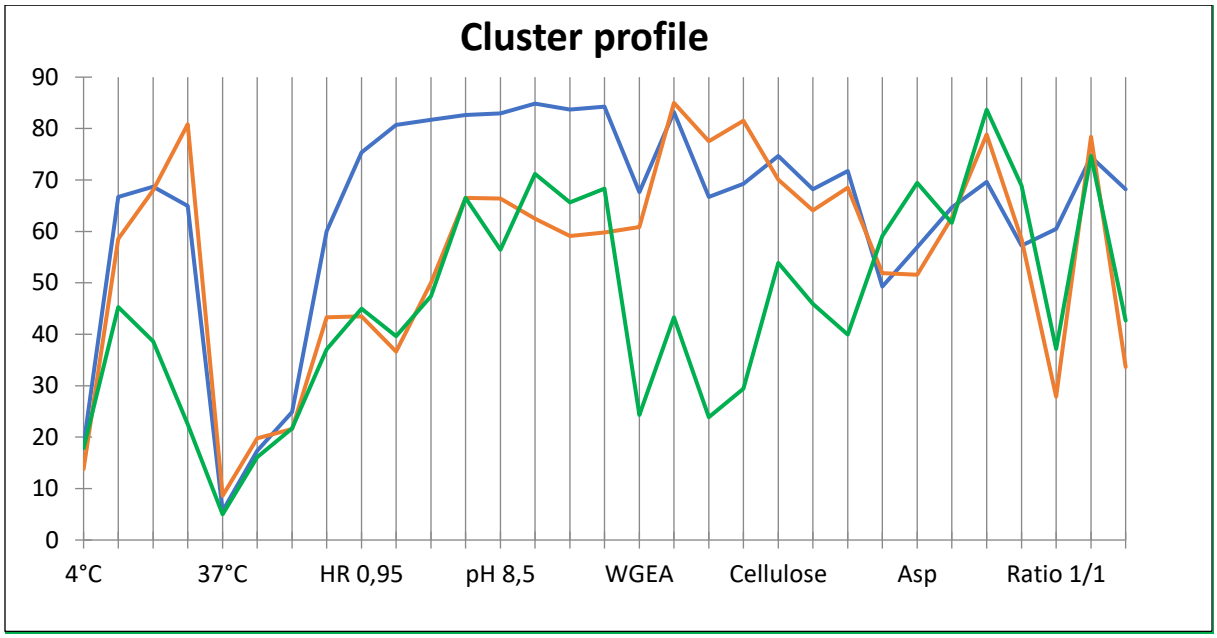


Figure 4.20. Profile of the classes of *Fusarium* strains according to the climatic and trophic parameters studied.

4.5. Discussion

Nutritional and ecological parameters play a significant role and are conducive to fungal growth, metabolism and pigment production in the field. In this work, we attempted to determine the impact of several factors, such as temperature, nutrients, pH, salinity, drought and relative humidity on the physiological processes of Fusaria. The results of this study show that Czapeck's agar was the appropriate medium for mycelial growth of *Fusarium* strains. This correlates perfectly with the results reported by Mohsen *et al*. (2016), who mentioned that *F. sacchari*, *F. globosum* and *F. proliferatum* showed faster mycelium growth on CDA, which provides the necessary nutritional requirements for the mycelial growth, such as sodium nitrate as a source of nitrogen, sucrose as a source of carbon and potassium phosphate as phosphorus (Mohsen *et al*., 2016). Similar study was performed by (Farooq *et al*., 2005), which states that the optimal media for mycelial growth of Fusarium wilt are CDA and CSMA. Differences may exist between *Fusarium* species as reported by Dikkar and Deshmukh (2003) who concluded that PDA is the most suitable for the growth of *F. oxysporum*. Equally, Khan *et al*. (2012) and Benaouali *et al*. (2014) also obtained the best growth and sporulation on the PDA medium.

Several pathogens naturally use nutritional factors like organic and inorganic matters, mineral salts and vitamins. The one-factor-at-a-time method in basal culture medium has been applied to assess the effects of various growth factors that can contribute to a better understanding of the population dynamics of pathogenic fungi in soil and other habitats.

Fungi had the innate versatility advantage over other microorganisms in being able to develop on a range of organic and inorganic carbon sources as substrates, and use them to produce cellular energy (Moore-Landecker, 1996). Screening all the carbon and nitrogen sources in this study, cellulose was the optimal carbon source, while the peptone was the most optimal nitrogen source for mycelial growth. The *Fusarium* strains tested develops faster on polysaccharide and disaccharide than on monosaccharide. The preference of Cellulose which is a homopolymer of glucose could be due to the activity of cellulase enzyme, which involved in its hydrolysis into glucose in abundance in the soil or other habitats that can be easily metabolised by fungi for energy production leading to maximum mycelial growth. Although peptones are water-soluble protein hydrolysates, containing peptides, amino acids, and inorganic salts as well as other compounds, such as lipids, vitamins, and sugars (Franěk *et al*., 2000). Our findings outlined above are in close agreement with those reported by Tang *et al.* (2022) found soluble starch and peptone as most suitable carbon and nitrogen source for *F. avenaceum* Charlie 779. Furthermore Siddeque *et al*. (2012) reported that peptone and sucrose as most suitable nitrogen and carbon source for Foc. On the other hand, Khan *et al*. (2012) recorded glucose and alanine as the most suitable carbon and nitrogen sources for *Foc* growth. Sucrose was also considered the best carbon source for *Fusarium solani* (Ramteke and Kamble, 2011). According to Benaouali *et al*. (2014), peptone was the best source of nitrogen for all the isolates of *F. oxysporum*. Further, Farooq *et al. (2005)*, showed that asparagine was observed as an excellent nitrogen source for growth of *F. oxysporum.*

The production of pigments by microorganisms is regulated by nutritional composition of the medium and other culturing conditions in which they are grown. The most significant variation in pigmentation was seen on medium with peptone, while media with amino acids produced lighter pigmentations. This closely conforms to the findings of Pradeep *et al*. (2013), who noted that peptone and yeast extract served as appropriate nitrogen sources for pigment production of *F. moniliforme* KUMBF1201. Boonyapranai *et al*. (2008) obtained a similar result with *F. verticillioides.*

Carbon and nitrogen (C:N) balance sustain optimal plant development, fungal growth, sporulation, and soil microbial metabolisms. Here, *Fusarium* spp. grew better when the C:N ratio was 10:1. Our results are in line with Jo *et al*. (2006), who observed that the optimum C:N ratios for mycelial growth of *Phellinus* spp. are 10:1 and 5:1. Various fungi have various C:N ratios. Lower and higher concentrations of carbon source in C:N ratio leads to reduce mycelial growth of *Fusarium* spp. This finding concurred with Shim *et al*.

(1997) who mentioned that the increased concentration of glucose in the C:N ratio was thought to be the cause of the decrease in mycelial growth of *Grifola umbellata*. In contrast, their observations disagreed with those of FusBo6.12 strain, which behaved strongly to low carbon source concentrations.

The incubation temperatures caused remarkable effects on the vegetative growth and sporulation of fungal species. The present study showed that the *Fusarium* spp. grows well at temperature range of 22 - 28°C, while a very slight growth was observed at 4°C, which can be attributed to the slowing down of fungal metabolic activities responsible for the ingestion of nutrients necessary for growth (Mensah-Attipoe and Toyinbo, 2019). No growth was recorded in any of the isolates evaluated at 37°C, which could be attributed to denaturation of some critical enzymes like glucosidase and fructosidase (Rehman *et al*., 2009). They may vary from isolate to isolate belonging to diverse ecological zones. Benaouali *et al*. (2014), demonstrated that the best temperatures of *Fusarium* growth were 23°C and 28°C. Tang *et al.* (2022) showed that *F. avenaceum* Charlie 779 grows well at temperature ranged from 15°C to 25°C. These results are also consistent with those of Kim *et al*. (2001) who found that the optimum temperature was at 26°C for all tested isolates *Fol* race1 *Fol* race 2 and *Forl.* and the growth of *F. oxysporum* f.sp *vanilla* was maximum at 25°C (Gangadhara *et al*., 2010), and Farooq *et al*. (2005) indicate at 25°C and 30°C, *Fusarium oxysporum* f. sp. *Ciceri* attained the maximum growth and decline above 35°C and drastically reduced below 15°C, however, no growth observed at 5°C. Similarly, Groenewald *et al.* (2006) revealed that the optimum temperature of *Fusarium oxysporum* f.sp. *cubense* was 25°C for almost all isolates and no growth was detected at 5 and 40°C for any isolate evaluated, while very little growth was registered at 10 and 35°C. The experiments of Popovski and Celar, (2013) mentioned that the optimal growth occurred at 25°C and 20-25°C for *F. graminearum* and *F. culmorum* respectively.

Climate (available water, extreme drought, as well as fluctuations of humid/dry cycles) is the most crucial agroecosystem factor affecting the phases of the life cycle of fungal disease and their capacity to colonize crops and survive (Paterson, 2006). In our study, significant growth was obtained at relative humidity levels of 95% and 100% respectively, whereas low growth was recorded with all isolates tested at relative humidity levels of 50% and 75%. Our findings clearly underscored the effect of relative humidity on mycelial growth of *Fusarium* spp. Deepthi *et al*. (2022) found that 96% of relative humidity favoured the growth of *Fusarium proliferatum* MYS9. Benaouali *et al*. (2014) noticed that *Fusarium oxysporum* f.sp *radicis lycopersici* grew well with a rate of humidity ranging

from 74 to 80%. According Baiyewu and Amusa (2005), the effect of temperature and relative humidity on pawpaw fruit rot in South-Western Nigeria, the relative humidity for the greatest rot growth of *Fusarium moniliforme* documented was between 60-80%, and Choi *et al*. (2015) noted at 97% RH, the population of *F. graminearum* increased significantly. Further, Choudhary *et al*. (2017) had seen that 100% relative humidity was optimum for growth of *Alternaria alternata* while low mycelial growth at 50% relative humidity.

Like other groups of soil borne fungi, the *Fusarium* has also its own preferences of pH. The present investigation revealed that pH 7 or 8.5 were the most suitable for the vegetative growth of *Fusarium* mycelia and the lowest radial growth of *Fusarium* spp. was recorded at pH 4.5. The present findings are in confirmation to those reported by Siddeque *et al.* (2012) found that the *Foc* produced maximum dry mycelial weight at pH 6.5 and also with reported by Khan *et al*. (2012) who noticed that pH 6.5-7.0 was the best for maximum growth of *Foc.*

This slight discrepancy in the reported results may be explained by the genetic differences found in the different strains of *Fusarium*. The minimum linear growth rates for *Fusarium* strains were recorded at pH 4.5. The most likely reason of this growth decline is the reduction in its enzymatic activities (Abdel Aziz *et al*., 2018).

In the current study, growth of most *Fusarium* isolates decreased with increasing salt concentrations. In line with findings of the present study, the endophytic fungi isolated from leaf and root as well as seeds from the salt-sensitive IR-64 variety and salt-tolerant Pokkali rice varieties also exhibited a decreased growth rate with increasing concentrations of NaCl (Sampangi-Ramaiah *et al*., 2020). Furthermore, our results indicated above revealed that ten of the isolates, FusBi1, FusBi15, FusBi2, FusBi21, FusBo28, FusBo35, FusBo50, FusBo59, FusBi7 and FusBo6.12 are extremely saline tolerant. Several reports have referenced endophytic fungi which are tolerant to high salt concentrations (Dastogeer *et al*., 2018; Sampangi-Ramaiah *et al*., 2020; Badawy *et al*., 2021).

4.6. Conclusion

The present study was designed to determine how different normal and stress conditions, individually, have varied regulatory patterns on the growth and metabolome of *Fusarium* spp. isolated from diseased durum wheat seeds and ears. It is likely that several genetic functions are included in these regulatory schemes. The most obvious finding to emerge from this study is that physiological behaviour differs among *Fusarium* isolates and

species in response to climate change. Further research is also needed to conduct on the *Fusarium* species responsible for Fusarium wilt in durum wheat to assess the combined effects of multiple environmental factors to better understand their behaviour. This would promote their use in large-scale control strategies against this fungus that is harmful to wheat crops.

5. *Fusarium* species associated with wheat head blight disease in Algeria: effects of triazole fungicides

5.1. Abstract

Fusarium head blight is an important disease of durum wheat which requires several fungicide treatments of seeds to achieve satisfactory control. The current study was carried out to evaluate commercially available fungicides *in vitro* for their efficacy against eighteen *Fusarium* spp. isolates collected from different fields in the north-eastern part of Algeria. Antifungal activity of fungicides shows that all triazoles tested have proven their effectiveness in inhibiting the mycelial growth of various strains of *Fusarium* tested. However, their sensitivity varies between them significantly (p<0.05) depending on the dose applied and period of exposure to each fungicide. The results showed that tebuconazole (Raxil and Tebuzole) and the combination fludioxonil + difenoconazole greatly reduced the mycelial growth of *Fusarium* isolates by 84.31%, 82.94%, 81.33%, respectively, as compared to difenoconazole alone (73.16%) at the recommended dose after five days of exposure. Regarding their effect on conidia germination, tebuconazole was more effective than fludioxonil + difenoconazole, which leads to deformation of cell wall structure and fragmentation of conidia. These results will provide useful information to select suitable fungicides for seed treatment and management of wheat head blight disease.

5.2. Introduction

Wheat is one of the major cereal crops produced worldwide with an output of 785 million tons (MT) in 2023 (FAO, 2023). Durum wheat (*Triticum durum*) takes a strategic place in the food system and national economy of Algeria with a production of 2.5 MT in 2021 (FAO, 2022). Several abiotic and biotic stressors may reduce this production. Among them, Fusarium head blight (FHB) is one of the most economically destructive diseases affecting cereal production worldwide (Goswami and Kistler, 2004; Wegulo *et al*., 2015). Infected grains become shrivelled and discoloured (white and/or pink), and premature bleaching and death of spikelets or entire heads may occur (Petronaitis *et al*., 2021).

Generally, up to 19 species in the genus *Fusarium* have been reported as causing FHB disease of wheat (Liddell, 2003), constituting a complex of toxigenic pathogens belonging to the genus *Fusarium* and the non-toxigenic genus *Microdochium* (Nielsen *et al*., 2011). Among different species causing FHB, *F. graminearum* is regarded as the most common causal agent worldwide because of its extensive occurrence and aggressiveness (Goswami and Kistler, 2004; Kazan *et al*., 2012). However, other causal agents are less commonly

reported, such as *F. poae*, *F. cerealis* and *F. equiseti*, and to a lesser degree *F. oxysporum*, *F. verticillioides* and *F. solani* (Bottalico and Perrone, 2002). Additionally, different regions may have different dominant FHB-causing species. For example, in Canada, *F. avenaceum* was the main causal agent of FHB in durum wheat (Tittlemeier *et al*., 2013), while *F. asiaticum* is the main FHB pathogen present in Asia (Ueda, 2007; Zhang *et al*., 2012). In Algeria, the FHB species *F. culmorum* was the most frequent and aggressive species on wheat seedlings (Abdallah-Nekache *et al*., 2019). The various FHB causal agents affect grain quality by accumulation of various mycotoxins, which cause health risks to both humans and animals. Aside from the health risk posed by mycotoxins, FHB has a double negative effect on returns to the producer through yield loss and reduced price for diseased commodity, reaching 52% of durum wheat yield losses in Australia, 50% in USA, 46% in Iran and 44% in Tunisia (Petronaitis *et al*., 2021). In recent decades, market discounts in the USA extend from USD 1.84 to 3.67 per tonne per 0.5 ppm of DON in grain (Dahl and Wilson, 2018).

According to new strategies of integrated pest management (IPM), many agronomic, genetic, biological tools, as well as agricultural practices, are now available to protect or restrict fungal diseases and related mycotoxin accumulation. The most effective control methods to minimize FHB impact are fungicide treatments (Malbrán *et al*., 2020), while anthesis applications can also be efficient (Rojas *et al*., 2020), and the use of resistant cultivars (Willyerd *et al*., 2012). Currently, chemical control of fungal pathogens can be achieved by several fungicides with different target sites, which are distinguished by their mode of action. The most recent target site fungicides are succinate dehydrogenase inhibitors (SDHIs), as well as the well-known phenylpyrroles (PP fungicides) that affect the fungal osmotic signal transduction cascade. There are also pathogen osmoregulators (fludioxonil is the best-known compound), benzimidazole carbamates and demethylation inhibitors (DMI) which affect sterol biosynthesis in membranes (Masiello *et al*., 2019).

Nowadays, triazoles are the most important fungicides applied in FHB control in the main wheat producing countries (Becher *et al*., 2011), likewise in Algeria. FHB is best monitored with triazole fungicides (Paul *et al*., 2008; Nakajima, 2010; Paul *et al*., 2010) which inhibit the cytochrome P450 sterol 14α-demethylase (CYP51), an enzyme required for ergosterol biosynthesis, causing fungal membrane structure to be disrupted (Ma and Michailides, 2005). Among triazoles, metconazole and tebuconazole are widely employed active substances to suppress FHB symptoms (Kotowicz *et al*., 2014), while

difenoconazole, as well as other DMI fungicides, have strong activity in controlling plant pathogenic fungi, including *Fusarium* species (Suty-Heinze and Dutzmann, 2004). The increasing use of triazole fungicides for FHB control has led to an emergence of resistant fungal pathogens, which have been recorded in populations of many major phytopathogenic fungi, including *Botrytis cinerea* (Stehmann and De Waard, 1996), *Venturia inaequalis* (Köller *et al*., 1997), *Blumeria graminis* f. sp. *tritici* (Godet and Limpert, 1998), *Mycosphaerella graminicola* (Mavroeidi and Shaw, 2005), *Colletotrichum cereale* (Wong and Midland, 2007), and *F. graminearum* (Yin *et al*., 2009). Studies associate decrease in DMI sensitivity to mutations in and over expression of the cyp51 gene (Leroux *et al*., 2007; Yin *et al*., 2009). Hence, determining the pathogenic population sensitivity to the most commonly used fungicides in disease control is an initial phase in developing an anti-resistant strategy (Lu *et al*., 2012).

For this reason, the present study aimed to evaluate *in vitro* the sensitivity of FHB isolates occurring on durum wheat to four commercial products containing difenoconazole, fludioxonil and tebuconazole, currently used for wheat seed treatment in Algeria. The efficacy of fungicides at different doses and over different exposure periods on *Fusarium* spp. was tested *in vitro* in solid medium to evaluate the inhibition of mycelial growth, and in liquid medium to examine their effects on spore germination.

5.3. Material and methods

5.3.1. Fungal material

Eighteen *Fusarium* isolates were isolated from FHB-symptomatic durum wheat grain samples and ears collected from various north-eastern provinces of Algeria. The set of isolates was identified in the previous chapter 1 and is codified as follows: *F. clavum* (FusBi8, FusBi1, FusBo25, FusBo28, FusBo49, FusBi2), *F. culmorum* (FusBo50, FusBo59), *F. microconidium* (FusBo26), *F. avenaceum* (FusBi7, FusBi21), *F. tricinctum* (FusBi6), *F. solani* (FusBo35), and *F. acuminatum* (FusBi23, FusBo33, FusBi15, FusBo11.5, FusBo6.12).

5.3.2. Fungicides used in *in vitro* assays

Four fungicides, registered for seed coating of cereals and belonging to DMIs: difenoconazole (Dividend 30 $g.L^{-1}$) and tebuconazole (Raxil 060 FS, Tebuzole 60 $g.L^{-1}$ FS) grouped as triazoles, and a mixture of fludioxonil (belongs to PPs) + difenoconazole (Celest Extra 25 $g.L^{-1}$ + 25 $g.L^{-1}$), were tested in this study. Based on the label dose

recommended by the manufacturers, we tested, for each fungicide, the manufacturers' recommended dose (D) and two lower dilutions, half (0.5 D) and decimal (0.1 D) as reported in Table 5.1. Stock solutions were prepared to obtain specific concentrations of the active ingredient.

Table 5.1. Fungicides tested in colony growth and conidial germination assays with *Fusarium* spp.

Fungicides		**Doses of a.i. tested (mg. L^{-1})**		
Active ingredients	**Trade names**	**D**	**0.5D**	**0.1D**
Difenoconazole	Dividend 30 FS	60	30	6
Fludioxonil + Difenoconazole	Celest Extra 25 + 25 $g.L^{-1}$	50+50	25+25	5+5
Tebuconazole	Raxil 60 $g.L^{-1}$ FS	30	15	3
Tebuconazole	Tebuzole 60 $g.L^{-1}$ FS	30	15	3

a.i. : active ingredient.

5.3.3. Effect of fungicides on mycelial growth of *Fusarium* isolates

The purpose of this experiment was to determine the efficacy of four fungicides and the behaviour of eighteen *Fusarium* strains based on mycelial growth *in vitro*, using the poisoned food technique (Nene and Thapliyal, 1993) and potato sucrose agar (PSA) as the basic culture media. Based on the active ingredient, appropriate amounts of each fungicide were determined and aseptically added to the sterilized and cooled (50°C) PSA medium to obtain the required concentrations in conical flasks separately, which were thoroughly shaken before being poured into 8.5 cm sterile Petri dishes. Three plates per treatment and per replication were maintained for each fungicide and its target concentration, and PSA Petri dishes without fungicide were used as controls.

The prepared dishes were aseptically inoculated with 5 mm diameter fungal plugs taken from the border of one week old culture and incubated at 25°C for 15 days. The results were recorded on the 5^{th}, 10^{th} and 15^{th} day of incubation by measuring the average diameter (mm) of fungal colonies from two perpendicular diameters. The mycelial growth inhibition (MGI, %) was determined using the following formula (Askarne *et al*., 2012):

$$\text{MGI (\%)} = \frac{\text{Dc} - \text{Dt}}{\text{Dc}} x\ 100$$

where Dc is the diameter of colony in control, and Dt is the diameter of colony in treatment.

5.3.4. Effect of fungicides on conidia germination of *Fusarium* isolates

In order to achieve a concentration of spores equal to 1×10^5 conidia. mL^{-1}, necessary for testing the effects of fungicides on the germination of spores, several culture media were used. For strongly sporulating strains we used PSA, while for less sporulating strains we used spezieller nährstoffärmer agar (SNA), and carnation leaf agar (CLA). However, for weakly sporulating strains we used pine needle medium (Su *et al*., 2012) for 10 days at 25°C.

Conidia were then obtained by scrubbing each colony surface with 10 mL of sterile distilled water containing 0.1% (v/v) tween 20 (for better conidia separation) and then filtering the suspension through two layers of sterile muslin to remove hyphal fragments. The resulting conidia in suspension were counted in Malassez cells and adjusted to 1×10^5 conidia. mL^{-1}. In order to evaluate the effect of the fungicides on conidia germination, a modified method of Li *et al.* (2022) was applied, where solutions of three fungicides (fludioxonil + difenoconazole, and tebuconazole: Raxil and Tebuzole) at their recommended and half doses were prepared in potato dextrose broth (PDB). For each concentration, a fungicide aliquot (75 µL) was mixed with 75 µL of conidia suspension (~1×10^5 conidia. mL^{-1}) in a 96-well plate, in triplicate. Controls were performed with 75 µL of sterile PDB and 75µL of the conidia suspension. The prepared plates were incubated at 25°C for 18 h and then observed with an optical microscope at ×10 magnification (B-290 Series, Optika). Germination and conidia anomalies (especially in macroconidia) were evaluated in nine replicates (three wells per treatment and three microscopic fields per well). Conidia were counted as germinated when the germ tube length was equal to or longer than the spore diameter (Klosowski *et al*., 2018). Conidia germination inhibition (CGI, %) was calculated using the following formula:

$$\mathrm{CGI}(\%) = \frac{\mathrm{Nt} - \mathrm{Ng}}{\mathrm{Nt}} x\ 100$$

where Nt and Ng are the total number of conidia examined and total number of germinated conidia, respectively.

5.3.5. Statistical analysis

In order to further compare the effectiveness of fungicides included in the study, mycelial growth inhibition and conidia germination inhibition of *Fusarium* species were analysed for each fungicide and concentration using the analysis of variance (ANOVA). Means were separated using Tukey's new multiple range test B (P=0.05). The SPSS 25 software (IBM, 2017) was used for all data analysis.

5.4. Results

5.4.1. Effect of fungicides on mycelial growth of *Fusarium* isolates

The analysis of variance shows a very highly significant fungicidal effect at 5% threshold on the mycelial growth of *Fusarium* strains studied as a function of doses applied and periods of fungicide exposure (Table 5.2). This shows a highly variable behaviour between the *Fusarium* isolates included in this study with respect to the fungicides tested.

Table 5.2. Variance analysis of fungicide effects depending on *Fusarium* isolates, doses and exposure periods.

Source of variation	Sum of squares	Df	Medium square	F	Signification
***Fusarium* isolates**	296716.79	17	17453.93	251.88	0.000
Fungicides	88184.37	3	29394.79	424.20	0.000
Dose of fungicides	232544.28	2	116272.14	1677.95	0.000
Periods of exposure	5226.68	2	2613.34	37.71	0.000
Total	10762039.80	1944			

a. R-square = 0.919 (Adjusted R-square = 0.878)

5.4.1.1. Efficiency of fungicides against *Fusarium* isolates

The effects of different concentrations on mycelial growth of *Fusarium* isolates were studied, and the results of three doses used: namely the recommended dose, half the recommended dose and one tenth of the recommended dose, on inhibition of mycelial growth revealed a significant difference at 5% threshold (Figures 5.1, 5.2) and correlated positively with dose and exposure period to fungicides.

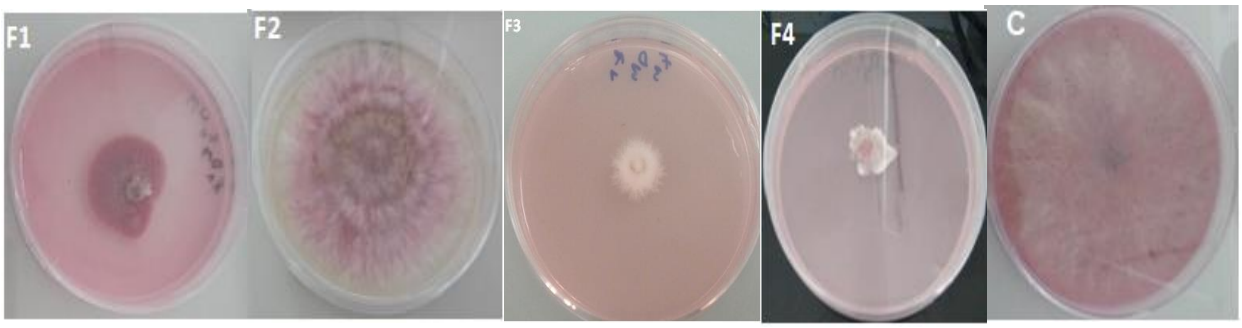


Figure 5.1. Effect of fungicides at 0.1D on the mycelial growth of *F. culmorum* (FusBo59 isolate). F1: Celest Extra, F2: Dividend, F3: Raxil, F4: Tebuzole, and C: Control.

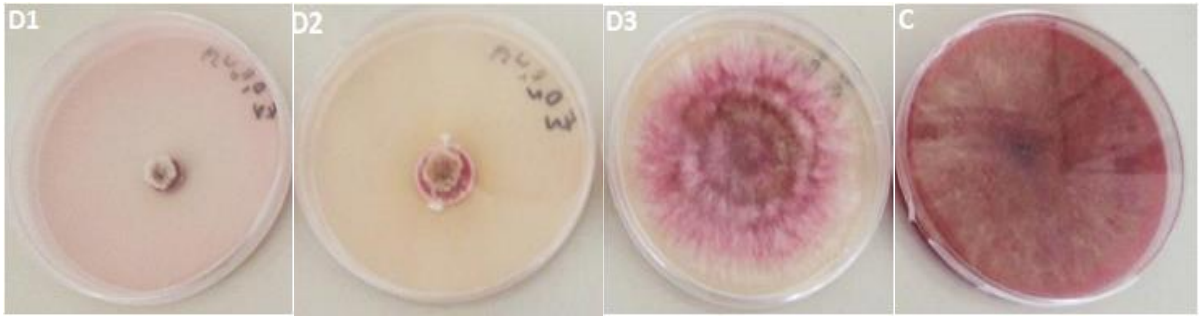


Figure 5.2. Effect of Dividend fungicide at different dose on the mycelial growth of *F. culmorum* (FusBo59 isolate). **D1:**D, **D2:**0.5D, **D3:**0.1D, and **C:** Control.

From the results obtained, we noted that the recommended dose of all fungicides was the most effective and reached its maximum after only 5 days of exposure of *Fusarium* strains to the fungicides, while a slight difference was observed between 5 and 10 days of exposure (Table 5.4).

By reducing the recommended dose by half, a slight difference in efficacy was observed. On the other hand, when the doses were divided by ten, the differences were quite noticeable. Thus, with difenoconazole (Dividend), we had a reduction in efficiency of around 48.18%, followed by tebuconazole, Raxil and Tebuzole, with 26.80% and 26.06%, respectively, and it was only equal to 19.21% with fludioxonil + difenoconazole (Celest Extra). It was also noted that the active ingredient tebuconazole, represented by the generic tebuconazole product Tebuzole and the innovative tebuconazole product Raxil, achieved effectiveness which was very close efficacy; we recorded inhibition rates of 82.94% and 84.31% by the recommended dose, respectively. Tukey's test B confirmed that they belong to the same group, proving that the generic product can have the same level of effectiveness as the innovative product.

Table 5.3. Mean effects of fungicides on mycelial growth of *Fusarium* isolates depending on doses tested.

Fusarium isolates	Recommended dose (D)				Half recommended dose (0.5D)				Tenth of recommended dose (0.1D)			
	Celest Extra	Dividend	Raxil	Tebuzole	Celest Extra	Dividend	Raxil	Tebuzole	Celest Extra	Dividend	Raxil	Tebuzole
FusBi1	86.25±1.77	85.33±1.02	59.80±8.20	87.24±1.16	73.70±1.77	83.23±1.53	58.42±7.94	82.23±1.64	81.95±3.46	81.11±2.05	75.18±5.95	78.96±4.07
FusBi11.5	58.60±2.63	76.94±0.83	65.20±7.99	64.91±2.02	81.00±2.47	72.87±3.71	65.50±7.86	63.99±2.79	75.85±5.28	28.84±6.21	60.39±9.18	52.82±4.27
FusBi15	68.86±1.07	34.94±1.53	73.61±4.95	82.47±1.52	76.99±1.37	36.58±1.98	70.45±5.21	50.44±3.20	22.24±2.64	61.88±3.69	68.78±7.94	38.70±4.73
FusBi2	86.24±2.98	70.70±3.19	72.40±4.74	83.01±1.09	84.41±3.82	43.36±5.95	69.91±7.02	74.40±2.14	76.47±6.62	26.86±9.24	68.05±11.14	85.16±2.89
FusBi21	84.00±4.48	71.04±7.24	64.01±9.32	94.12±0.00	6337±5.30	39.54±7.31	62.47±9.69	73.11±2.67	76.08±6.80	16.01±8.05	69.22±12.72	52.29±7.23
FusBi23	90.37±0.40	86.66±1.24	84.58±2.22	90.31±0.47	93.29±0.41	82.10±2.49	82.92±2.33	73.55±5.66	85.44±1.74	38.20±4.12	59.19±7.79	76.36±1.53
FusBi25	93.29±0.41	88.37±0.81	83.41±2.21	73.92±2.83	93.26±0.43	93.26±0.43	78.86±4.69	85.29±5.46	75.14±1.82	60.94±4.15	57.79±7.57	59.23±2.44
FusBi28	93.26±0.43	93.26±0.43	84.06±2.13	93.26±0.43	89.84±0.53	78.63±1.90	78.60±4.76	83.84±1.35	74.15±1.99	57.26±3.89	49.23±8.11	49.34±4.28
FusBi35	77.85±2.58	83.34±1.43	63.75±7.72	82.57±2.18	85.97±2.06	76.62±3.36	56.14±9.14	64.69±4.19	82.71±4.26	10.78±4.05	65.04±7.61	52.04±4.05
FusBi49	91.83±1.00	89.16±1.75	75.54±4.47	67.75±2.85	81.78±1.32	66.59±2.53	73.31±5.26	91.38±1.30	44.69±2.27	80.02±2.43	62.68±8.02	66.95±2.15
FusBi6	50.68±2.14	29.66±3.06	65.56±8.01	83.80±1.73	85.59±1.95	75.51±3.71	56.70±7.62	85.22±1.45	80.55±3.49	29.20±3.89	74.29±6.26	85.00±1.26
FusBi6.12	90.57±0.37	91.59±0.62	86.24±1.80	90.71±0.71	89.34±0.30	91.12±0.58	86.52±1.81	89.31±1.40	93.26±0.43	93.26±0.43	79.15±4.80	81.48±2.22
FusBi7	78.79±1.64	55.29±1.97	65.59±5.52	62.00±2.19	50.21±1.77	72.98±1.70	59.38±7.84	56.80±4.39	78.58±3.20	36.84±4.64	78.22±4.74	69.28±3.26
FusBi8	86.78±1.51	70.16±6.24	67.74±5.64	81.19±1.82	91.44±1.50	76.08±4.22	74.66±4.20	94.12±0.00	35.00±2.99	7.53±1.01	73.48±5.17	43.90±1.35
FusBo26	88.65±0.77	75.27±1.84	76.71±4.60	91.38±1.30	55.84±1.22	22.05±3.72	72.10±4.98	69.55±1.00	75.98±2.08	2.89±1.85	57.88±9.38	23.67±3.77
FusBo33	65.91±4.37	50.68±6.31	61.04±8.81	76.02±2.63	86.12±4.09	79.61±2.70	71.23±7.42	94.12±0.00	51.99±6.78	3.98±2.65	61.53±11.58	57.80±5.58
FusBo50	88.11±2.82	75.43±3.79	77.55±4.74	94.12±0.00	63.60±2.95	20.83±1.24	73.90±4.55	73.17±1.26	32.06±5.96	10.26±3.51	63.90±10.53	36.72±3.12
FusBo59	83.86±4.83	89.08±0.73	64.07±9.35	94.12±0.00	75.82±8.08	71.89±4.34	91.18±1.39	81.64±2.36	69.61±9.26	36.54±9.26	94.12±0.00	94.12±0.00
Mean	81.33±2.01	73.16±2.45	84.31±1.25	82.94±1.39	78.98±2.30	65.71±2.97	81.14±1.69	77.05±2.35	67.32±3.95	37.91±4.17	61.71±3.04	61.32±3.23

Results given in Mean ± SEM.

The lowest average inhibition of mycelial growth was recorded with the FusBi11.5 strain, 65.03%, which seems moderately resistant to the action of the fungicides tested. In contrast, the highest effectiveness of 93.28% was obtained with the FusBo28 strain after only 5 days of exposure to fungicides (Table 5.3). This isolate (FusBo28) was the most sensitive to all fungicides used, tebuconazole (Raxil and Tebuzole), difenoconazole, and fludioxonil + difenoconazole. On the other hand, FusBi6 was the most resistant strain to difenoconazole and to fludioxonil + difenoconazole with inhibition rates of 29.66% and 50.68%, respectively. FusBi7 was the most resistant strain to tebuconazole (Tebuzole) with an inhibition rate of 62.00%, while the most resistant strain to tebuconazole (Raxil) was FusBo11.5 with only 59.66%. Thus, a great variability was observed between *Fusarium* strains and it materialized by the formation of 15 groups through the statistical Tukey's B post hoc test.

Regarding the effect of exposure duration of *Fusarium* isolates to the fungicides tested, it appears that its extension did not in general increase their effectiveness through greater inhibition of mycelial growth. Overall, the rates of inhibition mycelial growth induced by the four fungicides after 5, 10 and 15 days of exposure varied in a non-significant manner. By way of example, we obtained the average inhibition rates of 84.82 ± 2.33% and 84.50 ± 2.54% after 5 days, 82.97 ± 2.67% and 82.72 ± 3.06% after 10 days, and 82.98 ± 2.95%, 81.86 ± 3.26% after 15 days of exposure to Raxil and Tebuzole, respectively (Table 5.4).

The differences observed in Table 5.4 are much more due to the dose effect and the behaviour of *Fusarium* isolates, and also to the depletion of nutrients in culture medium.

5.4.2. Effects of fungicides on spore germination of *Fusarium* isolates

The fungicides that showed the greatest efficiency in the mycelial growth test (fludioxonil + difenoconazole, tebuconazole: Tebuzole and Raxil) were also tested *in vitro* for their effect on conidial germination. The results showed a highly variable impact at 5% threshold between *Fusarium* isolates, fungicides and doses (Table 5.5).

The results of inhibition of conidia germination following treatment with fungicides revealed that tebuconazole (Tebuzole) was the most effective fungicide with 73.46 ± 1.18%, followed by tebuconazole (Raxil) with 69.753 ± 0.892%, even better than fludioxonil + difenoconazole, which only inhibited spore germination by 62.16 ± 0.789% at the recommended dose (Table 5.5). In addition, we noticed that the half dose proved to

Table 5.4. Mean effects of fungicides on mycelial growth of *Fusarium* isolates depending on exposure period.

Fusarium isolates	5 days exposure (P1)				10 days exposure (P2)				15 days exposure (P3)			
	Celest Extra	Dividend	Raxil	Tebuzole	Celest Extra	Dividend	Raxil	Tebuzole	Celest Extra	Dividend	Raxil	Tebuzole
FusBi1	87.10±1.17	76.53±8.11	89.63±1.12	87.29±2.39	83.01±2.91	68.19±8.98	84.12±2.26	87.21±1.89	80.00±4.17	77.67±6.14	76.91±4.13	84.03±2.55
FusBi11.5	53.24±4.20	71.79±5.33	84.26±3.30	82.33±4.94	56.66±3.53	68.75±5.40	85.02±2.94	83.31±3.77	57.57±3.42	66.79±5.20	83.17±2.11	75.19±4.25
FusBi15	57.32±3.09	62.50±4.70	83.28±2.11	75.83±2.90	58.15±3.62	67.10±4.95	84.20±2.96	80.51±4.37	57.77±4.04	71.15±5.96	84.22±3.31	83.51±4.35
FusBi2	88.60±1.30	85.05±2.28	90.21±1.71	90.59±0.88	87.11±1.18	76.48±8.10	89.18±1.25	87.80±2.50	72.73±5.63	63.45±7.67	77.66±4.32	75.24±4.38
FusBi21	85.20±2.37	70.06±9.77	89.18±1.25	86.94±2.48	62.77±4.67	60.45±6.10	77.61±2.86	76.35±3.48	61.47±4.58	82.98±2.05	91.17±1.02	89.84±1.23
FusBi23	88.25±1.27	81.03±6.42	88.75±1.85	89.06±2.20	81.97±2.97	73.69±8.20	83.50±2.17	86.88±2.10	84.32±2.66	78.81±5.45	82.19±1.73	86.09±2.37
FusBi25	84.24±2.64	83.25±1.69	80.32±0.95	85.56±2.41	81.95±4.27	76.60±6.61	77.34±4.23	79.45±4.07	63.40±6.11	63.38±6.34	78.95±3.40	75.56±3.85
FusBi28	88.71±1.33	86.10±2.05	89.55±1.75	90.82±0.95	91.78±0.70	87.39±2.22	90.52±1.88	92.17±1.02	90.87±0.89	86.21±2.08	90.52±1.88	91.89±1.00
FusBi35	58.62±3.67	60.02±6.07	79.24±3.14	75.19±2.89	57.52±3.39	63.19±4.57	83.29±2.76	78.68±4.03	56.44±4.35	74.92±5.41	82.57±3.26	84.30±4.70
FusBi49	81.95±4.27	74.84±6.53	78.13±4.40	78.47±3.74	84.21±2.76	78.14±6.22	79.35±3.23	84.66±2.70	74.21±5.93	70.14±6.44	81.50±3.77	77.47±4.79
FusBi6	60.23±4.80	61.30±6.41	78.50±3.26	74.71±3.68	57.96±3.30	65.75±5.63	83.41±2.03	77.51±3.54	56.34±3.82	63.91±4.86	82.25±2.48	75.55±4.34
FusBi6.12	88.45±1.26	79.33±6.22	89.84±1.65	89.06±2.20	85.66±2.29	64.16±10.02	86.95±2.08	85.02±2.39	71.74±5.83	62.23±6.51	79.31±3.48	73.45±3.96
FusBi7	84.47±2.60	84.51±1.81	79.35±3.23	85.57±2.90	85.09±2.35	64.05±10.01	89.64±1.37	86.61±2.45	82.45±3.16	73.04±8.04	83.18±2.17	86.88±2.10
FusBi8	83.62±2.62	70.24±9.49	85.83±2.12	87.44±1.97	78.26±5.35	72.67±6.77	79.03±4.67	76.46±4.34	67.53±5.60	64.62±6.90	79.34±3.48	76.01±3.64
FusBo26	84.15±2.76	69.80±9.36	85.43±2.25	87.21±1.89	86.42±2.33	63.94±9.98	88.65±1.69	85.77±2.51	74.24±5.94	73.10±6.82	79.41±4.67	75.16±4.32
FusBo33	72.01±5.72	66.89±6.50	76.81±4.33	74.47±4.21	62.20±4.80	62.81±6.49	77.05±2.71	75.12±3.47	55.02±4.94	76.50±4.63	86.17±3.44	88.81±1.22
FusBo50	90.34±0.80	86.13±2.06	90.06±1.83	91.43±0.97	83.81±2.53	83.68±1.88	77.63±2.80	84.70±2.74	71.18±0.00	86.54±0.03	90.29±1.25	90.88±1.53
FusBo59	87.82±1.29	80.94±6.39	88.36±1.74	89.06±2.20	81.75±4.29	75.75±6.39	76.95±4.14	80.73±3.63	56.66±3.81	69.92±5.64	84.93±3.14	83.54±4.03
Mean	79.13±2.62	75.02±5.62	84.82±2.33	84.50±2.54	75.91±3.18	70.71±6.58	82.97±2.67	82.72±3.06	68.55±4.16	72.52±5.34	82.98±2.95	81.86±3.26

Results given in Mean ± SEM.

be much less effective than the recommended dose, so that inhibition rate was reduced by more than half, particularly with fludioxonil + difenoconazole and tebuconazole (Raxil), giving only 27.558% and 33.582%, respectively. It is clear that the impact of fungicides on spore germination differs remarkably from their effect on mycelial growth in terms of efficacy and also in terms of ranking of the fungicides tested.

The results revealed that the fungicidal effect of Raxil is very limited on the FusBi1 strain by inhibiting only 3.060 ± 0.197% of spore germination (Table 5.5). However, the effect was very pronounced on other strains, such as FusBo26 and FusBo50 with 96.863 ± 0.265% and 96.010 ± 0.173% inhibition rates recorded with fludioxonil + difenoconazole and tebuconazole (Tebuzole), respectively. But it is even more pronounced with the FusBi15 strain, which achieved 100% inhibition rates noted with all fungicides tested. In the case of the FusBo33 strain, the results of fungicide effects on spore germination, unlike the mycelial growth test, should be taken with great caution because of its very low sporulation; despite testing several culture media that promote sporulation, we were unable to achieve the required concentration of 10^5 spore. mL^{-1}.

The microscopic examination of samples taken from spore suspensions of different *Fusarium* strains amended with fungicides revealed changes at the structural level compared to those that were not treated with fungicides (Figure 5.3a). Thus, tebuconazole (Raxil) caused deformation (Figure 5.3b) and fragmentation (Figure 5.3c) of conidia, while fludioxonil + difenoconazole only altered conidia through fragmentation (Figure 5.3c). It is also important to note that the effect of fungicides was notable in inhibiting germ tube elongation in all strains.

Table 5.5. Average results of fungicide effects on spore germination of *Fusarium* isolates.

Fusarium isolates	Fludioxonil + Difenoconazole		Tebuconazole (Tebuzole)		Tebuconazole (Raxil)	
	D	0.5D	D	0.5D	D	0.5D
FusBi1	20.030±0.296	13.423±0.377	40.883±6.135	6.907±0.395	3.060±0.197	0.000±0.000
FusBi11.5	25.707±0.083	15.663±0.055	93.437±0.353	86.140±0.598	71.357±0.930	25.460±0.501
FusBi15	100.00±0.000	79.767±0.319	100.00±0.000	96.863±0.388	100.00±0.000	85.023±0.481
FusBi2	27.830±0.514	0.000±0.000	86.937±1.104	88.677±0.333	85.627±0.468	0.000±0.000
FusBi21	54.383±2.420	1.233±0.291	100.00±0.000	98.147±0.437	84.287±0.563	25.993±1.442
FusBi23	96.723±0.435	67.750±1.432	100.00±0.000	98.167±0.218	100.00±0.000	86.907±0.619
FusBi25	29.647±0.229	22.510±0.797	32.410±1.170	41.817±2.160	48.863±3.924	21.430±0.785
FusBi28	84.820±0.765	0.950±0.137	74.917±1.468	67.637±1.050	80.960±0.626	8.800±0.478
FusBi35	81.033±0.112	10.787±0.166	54.850±0.430	29.380±0.993	42.193±0.578	8.967±0.357
FusBi49	89.153±1.460	23.027±1.320	53.787±2.670	11.573±0.109	75.460±2.162	7.563±0.114
FusBi6	96.977±0.535	31.020±0.806	100.00±0.000	100.00±0.000	98.890±0.262	75.427±0.500
FusBi6.12	59.160±0.374	45.157±0.215	76.343±1.197	73.400±0.759	76.663±0.425	44.560±0.246
FusBi7	97.187±0.366	14.617±0.432	93.027±0.111	99.470±0.125	97.090±0.172	62.900±0.294
FusBi8	24.247±1.311	12.917±0.528	89.303±0.840	89.303±0.840	58.153±1.529	18.220±1.188
FusBo26	96.863±0.265	97.870±0.248	89.073±0.489	91.703±0.245	97.907±0.143	94.550±0.431
FusBo50	90.620±0.330	18.660±0.292	96.010±0.173	91.667±0.447	95.843±0.324	7.807±0.431
FusBo59	30.060±1.314	27.570±0.276	26.880±1.694	24.583±1.997	24.773±0.362	17.753±0.192
Mean	62.16±0.789	27.558±0.599	73.461±1.18	67.142±0.788	69.753±0.892	33.582±0.619

Results given in Mean ± SEM.

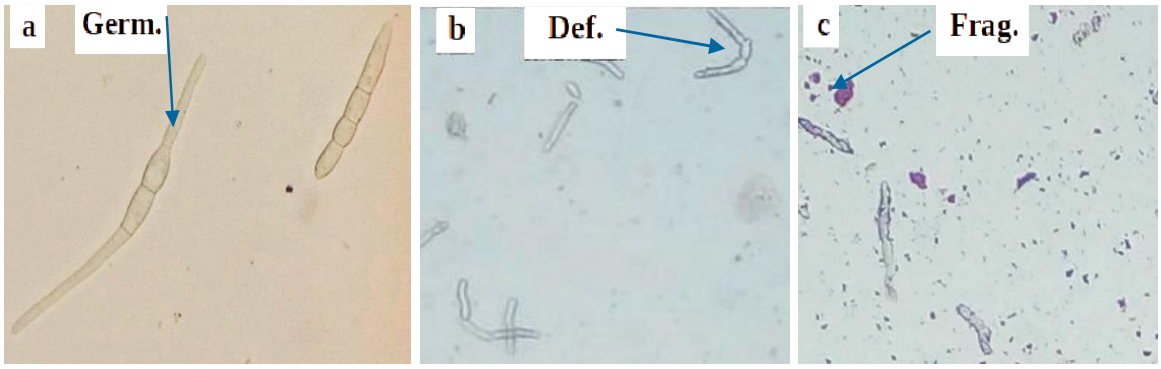


Figure 5.3. Effects of fungicides on the morphology of *F. avenaceum* conidia (FusBi7). Fungicides were mixed with conidia suspension at 25°C for 18 h and morphological differences were observed under optical microscope at ×10 magnification. (a) Conidia free of fungicide treatment germinated normally (Germ.); (b) Deformation (Def.) and distortion of conidia caused by tebuconazole (Raxil); (c) fragmentation (Frag.) of conidia caused by tebuconazole (Raxil) and fludioxonil + difenoconazole.

5.5. Discussion

The aim of this study was to assess *in vitro* the sensitivity of the fungal isolates causing FHB of wheat to the main fungicides currently used in several crops in Algeria. This provides critical information for disease control strategies.

This study offers new data on the sensitivity of most important *Fusarium* species associated with FHB of wheat to *Fusarium*-controlling fungicides that are necessary to limit crop losses. Triazoles are the most frequently applied fungicides for managing FHB because they are more effective than other active ingredients (Mateo *et al*., 2011, 2013; Haidukowski *et al*., 2012; Hellin *et al*., 2018). However, little is known about the impact of sublethal doses of these fungicides on the emergence of fungal resistances (Hellin *et al*., 2018). In fact, declining tebuconazole sensitivity has been reported in Germany (Klix *et al*., 2007) and China (Yin *et al*., 2009) because of the extensive use of fungicidal DMIs over the last 30 years.

With regard to the results obtained on the *in vitro* effects of fungicides, a significant effect of the tested commercial fungicides was recorded on radial mycelial growth of all *Fusarium* strains along the concentration gradient. Compared to the untreated control, all fungicides reduced the growth rates of all *Fusarium* strains, and the growth rates decreased as fungicide concentrations increased. Three fungicides (fludioxonil + difenoconazole, tebuconazole: Tebuzole and Raxil) were highly effective against all head blight isolates at

all concentrations. However, difenoconazole was a moderately effective fungicide. Generally, a positive correlation was observed between fungicide concentrations and inhibition of mycelial growth of *Fusarium* isolates. The inhibition rate reached its maximum after only five days of exposure, and stagnated at this level, while increase in exposure periods of *Fusarium* isolates to the fungicides tested did not influence mycelial growth inhibition.

In agreement with our results, the efficacy of fludioxonil in a mixture with difenoconazole against *F. solani* and *F. oxysporum* causing potato dry rot was demonstrated by Vatankhah *et al.* (2019). Fludioxonil action may be related to modification of the signal transduction pathways of *F. oxysporum*, which affects mycelial growth (Kim *et al*., 2007; Yang *et al*., 2011). A study conducted by Ochiai *et al*. (2002) also found that fludioxonil can disturb the CANIKI/COSI signal transduction pathway, which results in dysfunction of glycerol synthesis and inhibition of hyphae formation in *Candida albicans*. In contrast, difenoconazole alone was the least effective among the fungicides tested with only 73.16% inhibition rate. These results concur with those reported by Gxasheka *et al*. (2021), who found a slight decrease in mycelial growth of *F. graminearum* under the activity of higher concentrations of difenoconazole.

Decrease in mycelial growth due to tebuconazole, represented by the generic Tebuzole or the innovative product Raxil, was similar to the results obtained by Bhimani *et al*. (2018), who found an 87% reduction in mycelial growth of *F. oxysporum* by tebuconazole at low concentrations. Gxasheka *et al*. (2021) studied the effects of fungicides on *Fusarium* species causing maize ear rot disease in China, and also found that tebuconazole reduced mycelial growth of *F. oxysporum* by 67% with its lowest concentration. This could be explained by inhibition of the cytochrome P450 sterol 14α-demethylase (CYP51), an enzyme required for ergosterol biosynthesis, causing fungal membrane structure to be disrupted, which inhibits fungal growth (Ma and Michailides, 2005).

As the fungicides used in this test had the same concentration of active molecules, the isolates and different species showed different sensitivities to the same fungicides, which is in agreement with other studies. For example, fludioxonil + difenoconazole had different efficacy against *F. solani* and *F. oxysporum* isolates (Vatankhah *et al*., 2019). Gxasheka *et al.* (2021) also found that the same concentration of tebuconazole and difenoconazole had different efficacy results against *F. graminearum* and *F. oxysporum* isolates. Differences

in the effectiveness of the same fungicide in inhibiting mycelial growth of different *Fusarium* species and strains could be due to genetic polymorphism (higher or lower sensitivity of a strain) (Falcão *et al*., 2011). According to Hellin *et al*. (2018), *F. culmorum* could adapt to triazole pressure by major transcriptome modifications in response to triazole fungicides, including overly expression of drug resistance transporter, and the same mechanism is expected to occur in other species. Fungicide efficacy is influenced by fungal species, strains, ecological factors, and interactions among these factors (Mateo *et al*., 2011).

In vitro efficiency of fungicides regarding conidial germination indicated a significant effect between the fungicides selected and *Fusarium* strains studied. Triazoles inhibit 14-α-demethylase from taking part in the synthesis of ergosterol, the most common sterol in fungal cell membranes (Ma and Michailides, 2005). According to Shcherbakova *et al.* (2020), triazole fungicides effectively prevent the growth of a wide range of plant pathogenic fungi. It is often assumed that they are unable to inhibit the germination of their spores with the same efficacy because fungal spores already contain ergosterol, which is consistent with the results we obtained for tebuconazole. However, fludioxonil in a mixture with difenoconazole showed a germination inhibition rate of 62.16%, contrary to the results obtained by Rosslenbroich and Stuebler (2000), who reported that fludioxonil inhibited spore germination, germ tube elongation, and mycelium growth of *Botrytis cinerea* by affecting the osmoregulatory signal transmission pathway of that fungus. Moreover, our data also showed that the active ingredient tebuconazole represented by the innovative product namely Raxil caused more fragmentation and conidial malformations of strains, such as FusBi7, FusBo59 and FusBo26, than fludioxonil + difenoconazole, which caused conidial fragmentation in the FusBi7 strain. Malformation of conidia can be explained by findings that ergosterol biosynthesis-inhibiting fungicides frequently cause hitting morphological malformations, and irregular thickening of the cell wall (Ramirez *et al*., 2004), which can sometimes progress to fragmentation of conidia. Another possible explanation for conidia fragmentation could be related to the additive chemical products that differ in innovated and generic products, which are added to fungicides to improve their activity. The results indicate that these fungicides also inhibited the germination of conidia through degradation of cell structures, and not only by inhibiting germ tube elongation. To our knowledge, this is the first time that conidial fragmentation caused by

the tested fungicides has been reported. This new finding has major implications on the management of Fusarium head blight.

5.6. Conclusion

It was concluded that *in vitro* effects of fungicides have revealed a range of inhibitory activities against *Fusarium* isolates responsible for durum wheat head blight disease, including inhibition of mycelial growth, germination of spores, elongation of the germ tube and breakdown of cellular structures. Furthermore, none of the tested *Fusarium* strains showed resistance to triazoles applied under *in vitro* conditions. Given the importance and the need to control *Fusarium* wilt of durum wheat, *in vivo* experiments are necessary to validate these results. The information provided by this study may be useful for selecting the best active molecules against FHB and contribute to the evolution of an effective management strategy for this disease.

General conclusion and perspectives

Over the last decade, Algeria's climate is conducive to the growth of the most interesting genera of mycotoxigenic fungi, including *Fusarium*, responsible for the FHB disease in wheat crops. However, limited data are currently available on the biodiversity of FHB pathogens in Algeria. Several studies have focused on *F. culmorum* as the major causative agent of FHB in wheat. Therefore, this work was undertaken with the aim of studying the different *Fusarium* species involved in the FHB disease complex in Algeria and, mainly, studying them at different scales.

A polyphasic approach combining morphological (macro and micromorphological) and phylogenetic analyses inferred from the combined data set of ITS and *TEF*-1α loci was adopted to resolve the phylogeny of *Fusarium* isolates recovered from FHB of durum wheat. Eighteen *Fusarium* spp. were detected and identified to seven species as *F. clavum, F. culmorum, F. microconidium, F. avenaceum, F. tricinctum, F. solani,* and *F. acuminatum*, with *F. clavum* was the most common agent (33.3%). Four distinct species complexes occurred among our isolates, FCSC represented by *F. microconidium*, FTSC included *F. avenaceum, F. tricinctum,* and *F. acuminatum*, FIESC represented by *F.clavum*, and FSAMSC included *F. culmorum*, indicating that a high *Fusarium* biodiversity is detectable in Algeria.

Our findings of pathogenicity tests revealed significant variation in aggressiveness between isolates and between species, with *F. avenaceum* FusBi7 characterised to be the most aggressive. Moreover, differences in the behavior of durum wheat genotypes toward pathogenic infections have emerged, exhibiting the Cirta variety as the most tolerant to *Fusarium* strain attacks. This knowledge will be helpful for breeding programs intended to enhance cultivar resistance to decrease yield losses and mycotoxin accumulation in Algeria. It is also worth noting that pathogen isolates recovered from symptomatic ears and kernels can cause wheat crown rot, implying that the two diseases are caused by the same etiological agent.

The potential mycotoxin profile of *Fusarium* isolates indicated that the FusBo59 strain (*F. culmorum*) has a high toxigenic ability to produce DON compared to the rest of the strains, with a maximum level equal to 7.128 $\mu g.kg^{-1}$ in the ELISA test and 373196.19 $\mu g.kg^{-1}$ by LC-MS/MS exceeding the EC limits (1750 $\mu g.kg^{-1}$) for durum wheat intended for human and animal consumption. *F. microconidium* isolate simultaneously produced DON and ZEA, suggesting that cereal-based foods are likely to be contaminated with multi-

mycotoxins compounds. Traces of T-2 were detected in all the strains reaching a maximum of 0.35 $\mu g.kg^{-1}$, with no anticipated adverse effects on human health or food safety.

Toxin analysis of durum wheat samples using the chromatographic method revealed 15-ADON was the most frequent in wheat grains (63.6%) than DON (18.2%) and 3-ADON (9.1%). Low prevalence (18.2%) of samples contaminated with zearalenone (ZEA) at average level (2 $\mu g.kg^{-1}$) not exceeding authorized thresholds (100 $\mu g.kg^{-1}$) was noted. T-2 was detected in only one positive sample at levels below the LOQ, strongly confirming the results of our assay for the ability of pathogen isolates to produce mycotoxins. In addition, *Aspergillus* mycotoxin AFB2 was also found at a mean concentration of 18.5 $\mu g.kg^{-1}$, above the EC limit (4 $\mu g.kg^{-1}$). This adds to food safety worries. Although there was no correlation between *Fusarium* strains pathogenicity and mycotoxins production. These findings have significant implications for understanding the mechanisms of host-pathogen interaction and pathogen control.

The study of the ecophysiological profile of potentially pathogenic species helps to understand their behavior in response to climatic and trophic factors and to characterize the environmental and nutritional conditions limiting their growth and their metabolome expression, which will be useful for effective prevention and control of *Fusarium* disease in the field. The results of this study showed that Czapek Dox Agar at 25°C, 95% of relative humidity, pH 7, 2.5 g. L^{-1} of salinity, cellulose as carbon source, peptone as nitrogen source and 10:1 of C:N ratio, recorded the optimal mycelial growth of the pathogen isolates.

Four DMI fungicides registered for seed coating in cereals were used at different concentrations *in vitro* to determine their efficacy on *Fusarium* strains growth using the poisoned food technique. The findings revealed that tebuconazole (Raxil and Tébuzole) and the combination fludioxonil + difenoconazole greatly reduced the isolates growth by 84.31%, 82.94%, 81.33%, respectively, as compared to difenoconazole alone (73.16%) at the recommended dose after five days of exposure. The lowest average inhibition of mycelial growth was found with the FusBi11.5 strain, 65.03%, which appears to be moderately resistant to the action of the fungicides tested. In contrast, the highest efficacy of 93.28% was observed with the FusBo28 strain after only 5 days exposure to fungicides. Hence, this isolate (FusBo28) was the most sensitive to all the fungicides used. In terms of their effect on conidial germination, tebuconazole (Tebuzole 73.46%, Raxil 69.75%) was

more effective than fludioxonil + difenoconazole (62.16%) at the recommended dose, resulting in cell wall structure deformation and conidia fragmentation. Furthermore, it is noteworthy that the fungicides tested significantly inhibited germ tube elongation in all strains. These results will provide useful insights in the selection of the best active molecules for seed treatment and management strategy for this disease.

This research has thrown up many questions in need of further investigation, which can be recapitulated as follows:

- Extend the study to a wider panel of *Fusarium* species responsible for FHB in durum wheat to better assess potential variations in pathogenicity and to likely detect other distinct mycotoxin patterns.
- Obtain data on the role of durum wheat in Algerian consumers' daily ingestion of mycotoxins for risk monitoring purposes.
- Study the factors that may affect mycotoxins production, which will be helpful in determining the best management strategy to limit such production.
- Understand the role of multiple mycotoxins detected in this study in defining the level of pathogenesis and virulence of *Fusarium* spp. on cereals.
- Assess the combined effects of multiple environmental factors on the different physiological processes of Fusaria, such as fungal growth and sporulation. This would promote their use in large-scale control strategies against this harmful pathogen of wheat crops.
- Given the importance and the need to control Fusarium wilt of durum wheat, *in field* experiments are necessary to validate the results of this study.
- Develop efficient strategies to predict and control this disease for food and feed safety purposes in Algeria.
- Search for bioresistance genes in *Fusarium* species towards triazole fungicides applied for seed treatment.

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Appendices

APPENDICES

Appendix 1. The most commonly used fungicides for FHB control in Algeria.

Nom commercial	Active ingredient	Group name	Chemical group	Target site of action	Doses of use	Reference
Falcon®	Spiroxamine + Tebuconazole + Triadimenol	Amines ("Morpholines") SBI: Class II/ DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I)	Spiroketal-amines/ Triazoles	Δ^{14}-reductase and $\Delta^{8} \rightarrow \Delta^{7}$ Isomerase in sterol biosynthesis/ C14-demethylase in sterol biosynthesis (erg11/cy)	0,8 $L.ha^{-1}$	(FRAC, 2006) (DPPTC, 2017) (Degani *et al*., 2022)
Bunazol 250	Tebuconazole	DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I)	Triazoles	C14-demethylase in sterol biosynthesis (erg11/cy)	1 $L.ha^{-1}$	(DPPTC, 2017) (Degani *et al*., 2022)
Celest xtra 050 fs	Fludioxonil+ difenoconazole	PP-fungicides (PhenylPyrroles) / DMI fungicides (DeMethylation Inhibitors) (SBI: Class I)	Phenylpyrroles / Triazoles	MAP/Histidine Kinase in osmotic signal transduction (os-2, HOG1)/ C14-demethylase in sterol biosynthesis (erg11/cy)	200 $mL.kg^{-1}$	(DPPTC, 2017) (Degani *et al*., 2022)
Horizon 250 Ew	Tebuconazole	DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I)	Triazoles	C14-demethylase in sterol biosynthesis (erg11/cy)	1 $L.ha^{-1}$	(DPPTC, 2017) (Degani *et al*., 2022)

Appendix 1. *Cont.*

Nom commercial	Active ingredient	Group name	Chemical group	Target site of action	Doses of use	Reference
Pink	Trifloxystrobine	QoI-fungicides (quinone outside inhibitors)	Oximino acetates	Respiration C3: complex III: cytochrome bc1 (ubiquinol oxidase) at Qo site (cyt b gene)	500 $mL.ha^{-1}$	(DPPTC, 2017) (Degani *et al*., 2022)
Dividend	Difinoconazole	DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I)	Triazoles	Sterol biosynthesis in membranes G1:C14-Dimethylase in sterol biosynthesis (erg11/cyp51)	100 $mL.qL^{-1}$	(DPPTC, 2017) (Degani *et al*., 2022)
Dividend Star	Difinoconazole + cyproconazole	DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I)	Triazoles	Sterol biosynthesis in membranes G1:C14-Dimethylase in sterol biosynthesis (erg11/cyp51)	100 $mL.qL^{-1}$	(DPPTC, 2017) (Degani *et al*., 2022)
Horizell 25 ec	Tebuconazole	DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I)	Triazoles	C14-demethylase in sterol biosynthesis (erg11/cy)	40-100 $mL.hL^{-1}$	(DPPTC, 2017) (Degani *et al*., 2022)

Appendix 1. *Cont.*

Nom commercial	Active ingredient	Group name	Chemical group	Target site of action	Doses of use	Reference
Acanto ® plus	Cyproconazole + picoxystrobin	DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I)/ QoI-fungicides (quinone outside inhibitors)	Triazoles/ methoxy-acrylate	Sterol biosynthesis in membranes G1:C14-Dimethylase in sterol biosynthesis (erg11/cyp51)/ Respiration C3: cytochrome bc1 (ubiquinol oxidase) at Qo site (cyt b gene)	0,5 $L.ha^{-1}$	(DPPTC, 2017) (Feng *et al*., 2020) (Degani *et al*., 2022)
Lamardor 400 fs	Prothioconazole + tebuconazole	DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I)	Triazoles	C14-demethylase in sterol biosynthesis (erg11/cy)	20 $mL.qL^{-1}$	(DPPTC, 2017) (Tian *et al*., 2019) (Degani *et al*., 2022)
Talent 25 ec	Tebuconazole	DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I)	Triazoles	C14-demethyl-ase in sterol biosynthes-is (erg11/cy)	40-100 $mL.hL^{-1}$	(DPPTC, 2017) (Degani *et al*., 2022)

Appendix 2

Appendix 2.1. Macroscopic characteristics of 18 *Fusarium* isolates from this study.

***Fusarium* strains**	**Characteristics on PDA medium**			
	Aerial mycelium	**Color**		**Pigmentation**
		Recto	**Verso**	
F. clavum FusBi8	moderate, floccose	white to beige	beige	/
F. tricinctum FusBi6	abundant, floccose	yellow to rose and white with a beige border	burgundy with a beige border	burgundy
F. clavum FusBi2	moderate, floccose	white with a beige border	beige	/
F.avenaceum FusBi21	extremely abundant, floccose	burgundy with yellow	burgundy with a pale rose border	burgundy
F. clavum FusBi1	moderate, floccose	white with a beige border	beige	/
F.avenaceum FusBi7	extremely abundant, floccose	white	yellow and rose with a white border	brownish to burgundy
F. acuminatum FusBi15	moderate, floccose	yellow and burgundy with a white border	burgundy with a white border	red
F. acuminatum FusBi23	moderate, floccose	yellow and burgundy with a white border	burgundy with a white border	red

Appendix 2. 1. *Cont.*

***Fusarium* strains**	**Characteristics on PDA medium**			
	Aerial mycelium	**Color**		**pigmentation**
		Recto	**Verso**	
F. culmorum FusBo50	extremely abundant, floccose	yellow and burgundy	burgundy	red
F. culmorum FusBo59	extremely abundant, floccose	burgundy and pale orange	burgundy to pale rose	red
F. clavum FusBo25	abundant, floccose	beige	beige and dark brown	pale to dark brown pigment where the colony contacts the agar
F. clavum FusBo49	abundant, floccose	beige	beige and dark brown	pale brown pigment where the colony contacts the agar
F. microconidium FusBo26	abundant, floccose	yellow and burgundy	burgundy	burgundy pigment where the colony contacts the agar
F. clavum FusBo28	abundant, floccose	white	beige	pale to dark brown pigment where the colony contacts the agar
F. acuminatum FusBo6.12	rare, floccose	rose and beige at the periphery	brown and beige at the periphery	red

Appendix 2.1. *Cont.*

Fusarium **strains**	**Characteristics on PDA medium**			
	Aerial mycelium	**Color**		**Pigmentation**
		Recto	**Verso**	
F. acuminatum FusBo33	rare, floccose	pale yellow and white at the periphery	yellow and pale rose at the periphery	/
F. acuminatum FusBo11.5	rare, floccose	rose to burgundy and white at the periphery	rose to burgundy and white at the periphery	red
F. solani FusBo35	sparse, floccose	white and pale rose at the periphery	rose to brown and white at the periphery	/

Appendix 2.2. Microscopic characteristics of 18 *Fusarium* isolates from this study.

***Fusarium* strains**	**Microconidia on CLA**					**Macroconidia on CLA**				**Chlamydospore**
	Abund-ance in the aerial mycelium	**Shape**	**Disposition (direct observa-tion of the culture)**	**Aspect of the conidio-phore**	**Conidio-genesis**	**General shape**	**Basal cell shape**	**Apical cell shape**	**Abundance**	
F. clavum FusBi8	rare	fusiform	singly	long, non-branched	mono-phialide	Dorsiventral curvature	foot-shaped	tapering	abundant	abundant, in chain, sphere and oval, intercalary and terminal
F. tricinctum FusBi6	abundant	fusiform	singly	moderate, non-branched	mono-phialide	dorsiventral curvature	foot-shaped	papillate and hooked	moderate	absent
F. clavum FusBi2	rare	fusiform	singly	long, non-branched	mono-phialide	the dorsal side more curved than the ventral	foot-shaped	hooked	rare	abundant, sphere, in chains, yellow, intercalary and termina
F.avenaceum FusBi21	rare	fusiform	singly	long, non-branched	mono-phialide	straight	foot-shaped	hooked	moderate	absent

Appendix 2.2. *Cont.*

***Fusarium* strains**	**Microconidia on CLA**					**Macroconidia on CLA**				**Chlamydospore**
	Abund-ance in the aerial mycelium	**Shape**	**Disposition (direct observa-tion of the culture)**	**Aspect of the conidio-phore**	**Conidio-genesis**	**General shape**	**Basal cell shape**	**Apical cell shape**	**Abundance**	
F. clavum FusBi1	rare	ovale	singly	long, non-branched	mono-phialide	the dorsal side more curved than the ventral	foot-shaped	blunt	rare	extremely abundant, in chains, sphere, yellow, intercalary
F.avenaceum FusBi7	rare	ovale	singly		mono-phialide	straight	foot-shaped	papillate and hooked	moderate	absent
F. acuminatum FusBi15	moderate	ovale	singly	moderate, non-branched	mono-phialide	the dorsal side more curved than the ventral	distinctly notched	blunt	rare	rare, singly, sphere and ovale, intercalary
F. clavum FusBo49	moderate	ovale	singly	branched	mono-phialide	dorsiventral curvature	elongated foot	tapering	rare	abundant, singly and in chains

Appendix 2.2. *Cont.*

***Fusarium* strains**	**Microconidia on CLA**					**Macroconidia on CLA**				**Chlamydospore**
	Abund-ance in the aerial mycelium	**Shape**	**Disposi-tion (direct observa-tion of the culture)**	**Aspect of the conidio-phore**	**Conidio-genesis**	**General shape**	**Basal cell shape**	**Apical cell shape**	**Abundance**	
F. acuminatum FusBi23	rare	fusiform	singly	non-branched	mono-phialide	straight, the dorsal side more curved than the ventral	foot-shaped	hooked and blunt	rare	absent
F. microconidium FusBo26	abundant	ovale	singly	short branching	mono- et poly-phialides	straight, the dorsal side more curved than the ventral	foot-shaped	papillate	rare	rare, singly and in chains
F.acuminatum FusBo11.5	sparse	fusiform	singly	branched	mono-phialide	straight	barely notched	blunt	very abundant	sparse, in chains

Appendix 2.2. *Cont.*

***Fusarium* strains**	**Microconidia on CLA**					**Macroconidia on CLA**				**Chlamydospore**
	Abund-ance in the aerial mycelium	**Shape**	**Disposition (direct observa-tion of the culture)**	**Aspect of the conidio-phore**	**Conidio-genesis**	**General shape**	**Basal cell shape**	**Apical cell shape**	**Abundance**	
F.culmorum FusBo50	/	/	/	branched	mono-phialide	the dorsal side more curved than the ventral	barely notched	blunt	abundant	abundant, singly and in chains
F.culmorum FusBo59	/	/	/	branched	mono-phialide	the dorsal side more curved than the ventral	barely notched	blunt	abundant	abundant, singly and in chains
F. clavum FusBo25	/	/	/	branched	mono-phialide	dorsiventral curvature	elongated foot	tapering	abundant	abundant, singly and in chains
F. clavum FusBo28	moderate	ovale	singly	branched	mono-phialide	dorsiventral curvature	elongated foot	tapering	abundant	abundant, singly, verrucose walls

/= absence of conidia

Appendix 2.2. *Cont.*

***Fusarium* strains**	**Microconidia on CLA**					**Macroconidia on CLA**				**Chlamydospore**
	Abund-ance in the aerial mycelium	**Shape**	**Disposition (direct observa-tion of the culture)**	**Aspect of the conidio-phore**	**Conidio-genesis**	**General shape**	**Basal cell shape**	**Apical cell shape**	**Abundance**	
F.acuminatum FusBo6.12	sparse	reniform	singly	branched	mono-phialide	straight	barely notched	blunt	abundant	sparse, in chains
F.acuminatum FusBo33	rare	reniform	singly	branched	mono-phialide	straight	barely notched	blunt	rare	sparse, in chains
F.solani FusBo35	abundant	ovale	false heads	non-branched	mono-phialide	straight	barely notched	blunt	abundant	abundant, oval, singly

Appendix 2.3. Sporodochia features of 18 *Fusarium* isolates from this study.

Fusarium Strains	PDA at 25°C			SNA at 25°C			CLA at 25°C		
	Dispsi-tion	**Color**	**Abund-ance**	**Disposi-tion**	**Color**	**Abund-ance**	**Disposi-tion**	**Color**	**Abun-ance**
FusBi8	3	transparent	1	2 1	transparent transparent	1 4	2 4	transparent pale rose	2 2
FusBi6	2	transparent	3	/	/	/	4	gray	4
FusBi2	3	transparent	2	2	transparent	2	2 4	transparent transparent	2 1
FusBi21	2	pale orange	3	2 1	transparent transparent	1 2	2 1 4	pale orange pale orange pale orange	3 4 3
FusBi1	2 1	transparent transparent	1 2	2	transparent	1	2 4	transparent transparent	1 1
FusBi15	5	pale orange	1	3	transparent	2	2	pale orange	3
FusBi23	1	pale orange	1	1	transparent	1	2	pale orange	4
FusBi7	2	pale orange	2	2 1	transparent transparent	2 3	2 4	pale orange pale orange	4 4
FusBo59	2	transparent	2	2 1 5	transparent transparent transparent	2 2 3	4 1	beige transparent	3 3
FusBo26	2 5 1	yellow transparent transparent	1 4 2	2 1	transparent transparent	1 2	4 2	orange transparent	4 2
FusBo49	3	transparent	1	2 1	transparent transparent	1 2	4	transparent	4

Appendix 2.3. *Cont.*

Fusarium Strains	PSA at 25°C			SNA at 25°C			CLA at 25°C		
	Disps-tion	**Color**	**Abund-ance**	**Disposi-tion**	**Color**	**Abund-ance**	**Disposi-tion**	**Color**	**Abundance**
FusBo50	1 2	transparent white	4 2	2 1	yellow transparent	2 2	4 2	orange transparent	4 4
FusBo25	2	transparent	2	2 1	transparent transparent	1 2	4	transparent	4
FusBo28	3 5	transparent transparent	1 2	1	transparent	4	4	transparent	4
FusBo6.12	5 1	transparent transparent	3 3	2	transparent	2	4	transparent	4
FusBo11.5	5 2	transparent transparent	1 2	2 1	transparent transparent	4 2	4 2	transparent transparent	4 4
FusBo33	5 3	transparent transparent	1 4	2 1	transparent transparent	2 3	4	transparent	4
FusBo35	5	cream	1	2 1	transparent transparent	3 3	2	gray	2

Disposition (1: Dispersed over the whole colony; 2: Concentrated in the center of the colony; 3: 1 and 2; 4: Around the carnation leaf; 5: At the periphery).

Abundance (1: Extremely abundant; 2: Abundant; 3: Moderately abundant; 4: Poorly abundant). /= absence of sporodochia.

Appendix 3

PDA medium (potato dextrose agar)

Potato	200 g
Dextrose	20 g
Agar	20 g
Distilled water	1000 mL
Or	
PDA	39 g
Distilled water	1000 mL

This medium was autoclaved at 121°C for 20 min. pH: 7 ± 0.2.

PSA medium (potato sucrose agar)

Potato	200 g
Sucrose	20 g
Agar	20 g
Distilled water	1000 mL

This medium was autoclaved at 121°C for 20 min. pH: 7 ± 0.2.

PDB medium (potato dextrose broth)

Potato	200 g
Dextrose	20 g
Distilled water	1000 mL

This medium was autoclaved at 121°C for 20 min. pH: 7 ± 0.2.

WGEA medium (wheat grain extract agar)

Wheat grain	32 g
Dextrose	20 g
Agar	20 g
Distilled water	1000 mL

This medium was autoclaved at 121°C for 20 min. pH: 7 ± 0.2.

CDA medium (czapek dox agar)

Sucrose	30 g
$NaNO_3$	3 g
$MgSO_4 \cdot 7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4 \cdot 7H_2O$	0.01 g
K_2HPO_4	1 g
KH_2PO_4	1 g
Agar	20 g
Distilled water	1000 mL

This medium was autoclaved at 121°C for 20 min. pH: 7 ± 0.2.

SNA medium (spezieller nährstoffarmer agar)

K2HPO4	1 g
KNO3	1 g
MgSO4.7H2O	0.5 g
KCl	0.5 g
Glucose	0.2 g
Sucrose	0.2 g
Agar	20 g
Distilled water	1000 mL

This medium was autoclaved at 121°C for 20 min. pH: 7 ± 0.2.

CLA medium (carnation leaf agar)

Sterile carnation leaf fragments	
Agar	20 g
Distilled water	1000 mL

Appendix 4. Data on *Fusarium* strains isolated from FHB durum wheat samples.

Isolate Code	Species	Variety	Category	Year of production
FusBi8	*F. clavum*	MBB	G4	2018
FusBi1	*F. clavum*	Bousselam	R1	2018
FusBo25	*F. clavum*	Waha	R1	2018
FusBo28	*F. clavum*	GTAdur	R1	2018
FusBi2	*F. clavum*	GTAdur	R1	2018
FusBo49	*F. clavum*	vitron	R1	2018
FusBo50	*F. culmorum*	vitron	R1	2018
FusBo59	*F. culmorum*	vitron	R1	2018
FusBo26	*F.microconidium*	Waha	R1	2018
FusBi7	*F. avenaceum*	Waha	R1	2018
FusBi21	*F. avenaceum*	Cirta	R1	2018
FusBi6	*F. tricinctum*	Cirta	R1	2018
FusBo35	*F. solani*	GTAdur	R1	2018
FusBi23	*F. acuminatum*	Bousselam	R1	2018
FusBo33	*F. acuminatum*	GTAdur	R1	2018
FusBi15	*F. acuminatum*	Waha	R1	2018
FusBo11.5	*F. acuminatum*	GTAdur	R1	2018
FusBo6.12	*F. acuminatum*	Bousselam	R1	2018