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Original scientific paper

Fusarium species associated with wheat head blight disease in Algeria: characterization and effects of triazole fungicides

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SUMMARY

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. The morphological and molecular characterization reveals the presence in wheat seeds of the main species complexes *F. acuminatum, F. equiseti, F. avenaceum, F. solani, F. culomorum, F. incarnatum-equiseti,* as well as *F. tricinctum* species complex and *F. chlamydosporum* species complex. 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 Tébuzole) 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.

Keywords: wheat, *Fusarium*, fungicides, toxicity

INTRODUCTION

Wheat is one of the major cereal crops produced worldwide with an output of 776.7 million tons over 2021-2022 (FAO 2022). Durum wheat (*Triticum durum*) takes a strategic place in the food system and national economy of Algeria with a production of 2.5 million tons 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 & 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* (teleomorph = *Gibberella*) 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 & 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 & 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 (Zhang et al., 2012; Ueda et al., 2007). 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 & 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 (Nakajima, 2010; Paul et al., 2010; Paul et al., 2008) which inhibit the cytochrome P450 sterol 14α-demethylase (CYP51), an enzyme required for ergosterol biosynthesis, causing fungal membrane structure to be disrupted (Ma & 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 & 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 & De Waard, 1996), *Venturia inaequalis* (Köller et al. 1997), *Blumeria graminis f. sp. tritici* (Godet & Limpert, 1998), *Mycosphaerella graminicola* (Mavroeidi & Shaw, 2005), *Colletotrichum cereale* (Wong & 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 *Fusarium* species 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* species 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.

MATERIAL AND METHODS

Sample collection and fungal isolation

After the harvest season 2017-2018, the CNCC (National Center for Seeds and Plants Certification and Control) of Setif state supplied sixty durum wheat samples (diseased seeds and ears) from six varieties, namely Bousselam, Mohamed Ben Bachir, GTAdur, Cirta, Waha and Vitron, collected from districts in the north-eastern parts of Algeria, including: Setif, Bordj Bou Arreridj (BBA), M'sila, Batna, Khenchela, Biskra and Mila. The samples were stored in paper bags at a temperature of 4°C until further use.

The 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 for 10 min and then thoroughly rinsed with sterile distilled water. After that, they were dried with sterile filter paper for 20 to 30 minutes under aseptic conditions. Surface disinfected seeds were plated on Potato Sucrose Agar (PSA) medium, seven to eight seeds per plate, and incubated at 25°C for 5-7 days. Different types of fungal colonies were observed on the PSA medium, but only typical colonies and conidia with *Fusarium* traits were selected, purified (using the single-spore technique) and then submitted to morphological features examination.

Morphological identification of *Fusarium* isolates

Preliminary identification of *Fusarium* spp. was carried out according to Leslie and Summerell (2006). The critical characteristics that were assessed included macroscopic traits on the PSA (growth rates, presence of aerial mycelium, colony appearance and texture, pigmentation on both top and reverse plates) and microscopic traits on Carnation Leaf Agar (CLA). The evaluation of microscopic criteria was done using a method suggested by the National Plant Protection Laboratory, France (LSV, 2008). Plates with *Fusarium* spp. cultures on CLA were first placed under a stereo microscope to observe sporodochia (disposition, color, abundance), and then two samplings were systematically carried out for observation in a drop of dye (lactophenol cotton blue). The first sampling consisted of collecting aerial mycelium, and noting the characteristics of microconidia (shapes size, abundance, conidiophore appearance, conidiogenesis). The second sampling consisted of collecting sporodochia for observation of macroconidia produced in sporodochium (shapes size, abundance). The presence and appearance of chlamydospores was studied from samples taken from the aerial mycelium of cultures on PSA.

Molecular identification of *Fusarium* isolates

Genomic DNA was extracted using 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) region was done using the primers 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) gene using the primers EF1/EF2 (5'-ATGGGTAAG GAG GACAAG AC-3'/5'-GGAAGTACCAGT GAT CAT GTT-3') (O'Donnell et al., 2000; O'Donnell et al., 2004; Proctor et al., 2009). PCR amplification was performed using the KAPA3G Plant PCR Kit (Kapa Biosystems, Boston, USA). PCR assay was carried out in the thermal cycler (T100™ Thermal Cycler; Bio-Rad, Irvine, CA) and PCRs were conducted in 25µl volume reactions containing 1x buffer, 2.0 mM$MgCl_2$, 0.2 mM each dNTPs, 0.3µM of each primer, 1U of HOT FIREPol® DNA Polymerase (Solis Biodyne) and 1µl 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: 50°C, 15 sec and for TEF-1α: 53°C, 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, following the company protocol. Subsequently, sequences were compared with those in the public databases (GenBank for rDNA-ITS for non-*Fusarium* species and FUSARIUM MLST for rDNA-ITS and TEF-1α for *Fusarium* species) and only similarity levels ≥ 99% were retained for identification of *Fusarium* isolates.

Fungicides used in *in vitro* assay

Four fungicides, registered for seed coating of cereals and belonging to DMIs: difenoconazole (Dividend 30g/l) and tebuconazole (Raxil 060 FS, Tébuzole 60 g/l FS)

grouped as triazoles, and a mixture of fludioxonil (belongs to PPs) + difenoconazole (Celest Extra 25g/l + 25g/l), 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 (0.5D) and decimal (0.1D) as reported in Table 1. Stock solutions were prepared to obtain specific concentrations of the active ingredient.

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 & Thapliyal, 1993) and Potato Sucrose Agar (PSA) as a 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 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 5th, 10th and 15th day of incubation by measuring the average diameter (in mm) of fungal colonies from two perpendicular diameters. The mycelial growth inhibition (MGI, %) was determined using the following formula (Askarne et al., 2012):

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

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

Effects 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 and Spezieller Nährstoffärmer Agar (SNA), and CLA for less sporulating ones (Leslie & Summerell, 2006). 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 Tébuzole) 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 :

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

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

Table 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 + 25g/l	50+50	25+25	5+5
Tebuconazole	Raxil 60 g/l FS	30	15	3
Tebuconazole	Tébuzole 60 g/l FS	30	15	3

a.i.: active ingredient

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.

RESULTS

Pathogen isolation and identification

Based on morphological characteristics of the fungal isolates (Leslie & Summerell, 2006) obtained from diseased seeds and ears, eighteen fungal isolates belonging to the *Fusarium* genus, disseminated in various proportions throughout the study areas, were revealed. Most of the fungal isolates were present in Mila District, 33.33%, followed by BBA, Batna and M'sila Districts, 22.22%, 16.66%, 11.11%, respectively. The lowest percentage, 5.55%, was in Setif, Khenchla and Biskra Districts.

Molecular identification was based on their rDNA-ITS region and the TEF-1α gene of all fungal strains isolated in this study, and they were successfully amplified with primers ITS1-ITS4/ EF1-EF2, which resulted in amplicons of 500 bp (Figure 1a) and 700 bp (Figure 1b), respectively.

This analysis showed that the isolated strains belonged to five species, namely *F. avenaceum* (FusBi7, FusBi21), *F. acuminatum* (FusBi15, FusBi23, FusBo11.5, FusBo6.12, FusBo33), *F. culmorum* (FusBo50, FusBo59), *F. equiseti* (FusBo25, FusBo28, FusBo49) and *F. solani* (FusBo35), and three complexes, including *F. incarnatum-equiseti* (FusBi8, FusBi1, FusBi2), *F. tricinctum* (FusBi6) and *F. chlamydosporum* (FusBo26) based on the sequences of rDNA-ITS region and TEF-1α gene for each of them, which were ≥99% similar reference sequences.

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 2). This shows a highly variable behaviour between the *Fusarium* isolates included in this study with respect to the fungicides tested.

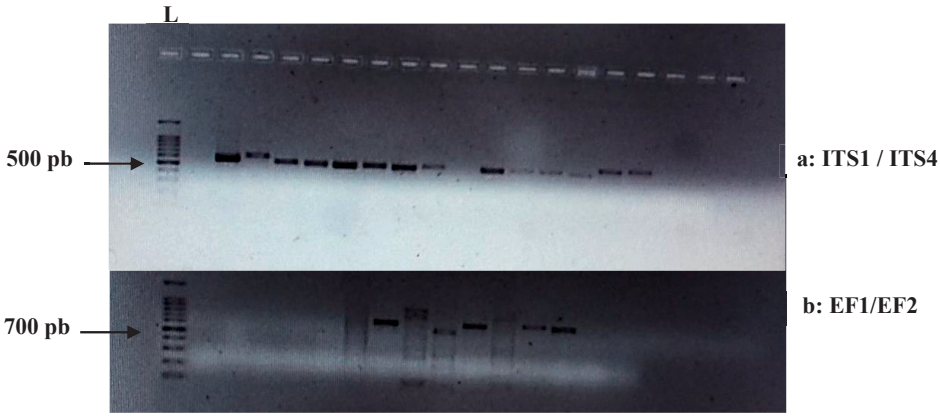

Figure 1. Electrophoresis products of amplified DNA of isolates of *Fusarium* spp. **a:** Amplification with universal primers ITS1/ ITS4, **b:** Amplification with universal primers EF1/EF2, **L:** Ladder.

Table 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)					

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 and correlated positively with the dose and exposure period to fungicides.

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 days and 10 days of exposure (Table 3).

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 Tébuzole and the innovative tebuconazole product Raxil, achieved effectiveness which was very close; 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.

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 3). This isolate (FusBo28) was the most sensitive to all fungicides used, tebuconazole (Raxil and Tébuzole), 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 (Tébuzole) 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.

Table 3: Mean effects of fungicides on mycelial growth of *Fusarium* isolates depending on doses tested

Fusarium isolates	Recommended dose (D)				Half recommended dose (O,5D)				Tenth of recommended dose (0.1D)			
	Celest Extra	Dividend	Raxil	Tébuzole	Celest Extra	Dividend	Raxil	Tébuzole	Celest Extra	Dividend	Raxil	Tébuzole
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	63.37±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±SE

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 of 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 Tébuzole, respectively (Table 4).

The differences observed in Table 4 are due much more to the dose effect and to the behaviour of *Fusarium* isolates, and also to the depletion of nutrients in culture medium.

Effects of fungicides on spore germination of *Fusarium* isolates

The fungicides that showed greater efficiency in the mycelial growth test (fludioxonil + difenoconazole, tebuconazole: Tébuzole 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).

The results of inhibition of conidia germination following treatment with fungicides revealed that tebuconazole (Tébuzole) was the most effective fungicide with 73.461±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). In addition, we noticed that the half dose proved to 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, while FusBo33 was particularly resistant to fludioxonil + difenoconazole and tebuconazole (Raxil), germinating only up to 14.443± 3.408% (Table 5). However, the effect was very pronounced on other strains, such as FusBo26 and FusBi15 with 96.863±0.256% and 96.010±0.173% inhibition rates recorded with fludioxonil + difenoconazole and tebuconazole (Tébuzole), respectively. But it is even more pronounced with the F usBi15 strain, which achieved

Table 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	Tébuzole	Celest Extra	Dividend	Raxil	Tébuzole	Celest Extra	Dividend	Raxil	Tébuzole
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±SE

100% germination in the presence of tebuconazole (Raxil). 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 low sporulation; despite testing several culture media that promote sporulation, we were unable to achieve the required concentration of 10^5 sp/ml.

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 2a). Thus, tebuconazole (Raxil) caused deformation (Figure 2b) and fragmentation (Figure 2c) of conidia, while fludioxonil + difenoconazole only altered conidia through fragmentation (Figure 2c). It is also important to note that the effect of fungicides was notable in inhibiting germ tube elongation in all strains.

Table 5: Average results of fungicide effects on spore germination of *Fusarium* isolates

Fusarium isolates	Fludioxonil + Difenoconazole		Tebuconazole (Tébuzole)		Tebuconazole (Raxil)	
	D	0.1D	D	0.1D	D	0.1D
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
FusBo33	14.443±3.408	13.130±3.098	14.443±3.408	13.130±3.098	14.443±3.408	13.130±3.098
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±SE

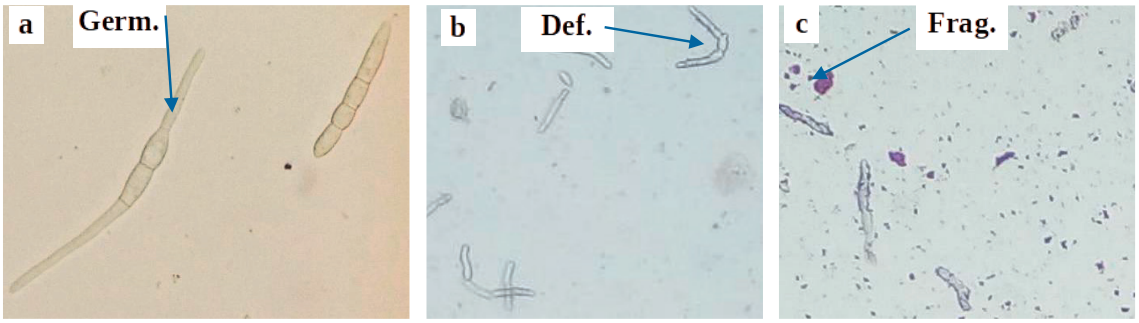

Figure 2. Effects of fungicides on the morphology of *F. avenaceum* conidia (FusBi7). Fungicides were mixed with conidia suspension at 25°C for 18 h and morphological differences were observed under optical microscope at ×10 magnification. (**a**) Conidia free of fungicide treatment germinated normally (Germ.). (**b**) Deformation (Def.) and distortion (**c**) of conidia caused by tebuconazole (Raxil); fragmentation (Frag.) of conidia caused by tebuconazole (Raxil) and fludioxonil + difenoconazole

DISCUSSION

The aims of this study were to identify the fungal species causing FHB of wheat and assess *in vitro* their sensitivity to the main fungicides currently used in several crops in Algeria. This provides critical information for disease control strategies. Identification of *Fusarium* isolates was performed with morphological and molecular techniques using PCR with primer sets. Some variation was found in the overall prevalence among species of the 18 *Fusarium* isolates collected in the study area. *F. acuminatum* was the most prevalent with a frequency of 27.7% of *Fusarium* isolates, followed by *F. incarnatum-equiseti* species complex and *F. equiseti* with a frequency of 16.6% each. The least commonly isolated species were *F. solani, F. tricinctum* species complex and *F. chlamydosporum* species complex with 5.5% frequency. *F. acuminatum* was the dominant species isolated from the head of wheat in north-eastern districts of Algeria which is quite equivalent to the results reported by Shikur et al. (2018) where it was the second most frequent species isolated from the crown of wheat in Turkey. However, *F. culmorum* was reported as the species most frequently isolated in other districts of northern Algeria (Abdallah-Nekache et al., 2019; Hadjout et al., 2022). But on the other hand, *F. graminearum* species complex was the predominant species isolated from heads of wheat in several other countries, including Iran (Sharifi et al., 2016) and Brazil (Pereira et al., 2021).

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 obtained results of *in vitro* effects of fungicides, a significant effect of the tested commercial fungicides was recorded on radial mycelial growth of all strains of *Fusarium* 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: Tébuzole 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 58.93% 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 Tébuzole or the innovative product Raxil, was similar in our study 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 & 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 & 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.

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.

REFERENCES

Abdallah-Nekache, N., Laraba I., Ducos, C., Barreau, C., Bouznad, Z., & Boureghda, H. (2019). Occurrence of *Fusarium* head blight and *Fusarium* crown rot in Algerian wheat: identification of associated species and assessment of aggressiveness. *European Journal* of *Plant Pathology, 154*(3), 499-512. https://doi.org/10.1007/s10658-019-01673-7

Askarne, L., Talibi, I., Boubaker, H., Boudyach, E.H., Msanda, F., Saadi, B... Ait Ben Aoumar, A. (2012). *In vitro* and *in vivo* antifungal activity of several Moroccan plants against *Penicillium italicum*, the causal agent of citrus blue mold. *Crop Protection*, *40*, 53-58. https://doi.org/10.1016/j.cropro.2012.04.023

Becher, R., Weihmann, F., Deising, H.B., & Wirsel, S.G.R. (2011). Development of a novel multiplex DNA microarray for *Fusarium graminearum* and analysis of azole fungicide responses. *BMC Genomics*, *12*, 52. https://doi.org/10.1186/1471-2164-12-52

Bhimani, M.D., Golakiya, B.B., & Akbari, L.F. (2018). Evaluation of different fungicides against fenugreek wilt (*Fusarium oxysporum* Schlecht.). *International Journal* of *Chemical* Studies, *6*(2), 29-34.

Bottalico, A., & Perrone, G. (2002). Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. *European Journal of Plant Pathology*, *108*(7), 611-624. https://doi.org/10.1023/A:1020635214971

Dahl, B., & Wilson, W.W. (2018). Risk premiums due to *Fusarium* head blight (FHB) in wheat and barley. *Agricultural Systems*, *162*, 145-153. https://doi.org/10.1016/j.agsy.2018.01.025

Falcão, V.C.A., Ono, M.A., de Ávila Miguel, T., Vizoni, E., Hirooka, E.Y., & Ono, E.Y.S. (2011). *Fusarium verticillioides*: evaluation of fumonisin production and effect of fungicides on in vitro inhibition of mycelial growth. *Mycopathologia*, *171*, 77-84. https://doi.org/10.1007/s11046-010-9339-9

FAO (2022). Food and Agriculture Organization of the UN. World Food Situation. Available online: https://www.fao.org/worldfoodsituation/csdb/en/ (accessed on 15 February 2022).

Godet, F., & Limpert, E. (1998). Recent evolution of multiple resistance of *Blumeria* (*Erysiphe*) *graminis* f.sp. *tritici* to selected DMI and morpholine fungicides in France. *Pesticide Sciences*, *54*(3), 244-252. https://doi.org/10.1002/(SICI)1096-9063(1998110)54:3<244::AID-PS818>3.0.CO;2-8

Goswami, R.S., & Kistler, H.C. (2004). Heading for disaster: *Fusarium graminearum* on cereal crops. *Molecular Plant Pathology*, *5*(6), 515-525. https://doi.org/10.1111/j.1364-3703.2004.00252.x

Gxasheka, M., Wang, J., Gunya, B., Mbanjwa, V., Tyasi, T.L., Dlamini, P., & Gao, J. (2021). In vitro effect of some commercial fungicides on mycelial growth of *Fusarium* species causing maize ear rot disease in China. *Archives of Phytopathology and Plant Protection*, *54*(11-12), 557-569. https://doi.org/10.1080/03235408.2020.1844531

Hadjout, S., Chéreau, S., Mekliche, L., Marchegay, G., Ducos, C., Boureghda, H.... Richard-Forget, F. (2022). Molecular identification of some *Fusarium* isolates and their chemotypes involved in fusarium head blight on Durum wheat in Algeria. *Archives of Phytopathology and Plant Protection*, *55*(4), 499-513. https://doi.org/10.1080/03235408.2022.2034363

Haidukowski, M., Visconti, A., Perrone, G., Vanadia, S., Pancaldi, D., Covarelli, L.... Pascale, M. (2012). Effect of prothioconazole-based fungicides on *Fusarium* head blight, grain yield and deoxynivalenol accumulation in wheatunder field conditions. *Phytopathologia Mediterranea, 51*(1), 236-246. https://doi.org/10.14601/Phytopathol_Mediterr-9401

Hellin, P., King, R., Urban, M., Hammond-Kosack, K.E., & Legreve, A. (2018). The adaptation of *Fusarium culmorum* to DMI fungicides is mediated by major transcriptome modifications in response to azole fungicide, including the overexpression of a PDR transporter (FcABC1). *Frontiers in Microbiology*, *9*, 1385. https://doi.org/10.3389/fmicb.2018.01385

IBM (2017). *IBM SPSS Statistics for Windows*, Version 25.0. Armonk, NY: IBM Corp. Available at: https://hadoop.apache.org

Kazan, K., Gardiner, D.M., & Manners, J.M. (2012). On the trail of a serial killer: recent advances in *Fusarium graminearum* pathogenomics and host resistance. *Molecular Plant Pathology*, *13*(4), 399-413. https://doi.org/10.1111/j.1364-3703.2011.00762.x

Kim, J.H., Campbell, B.C., Mahoney, N., Chan, K.L., Molyneux, R.J., & May, G.S. (2007). Enhancement of fludioxonil fungicidal activity by disrupting cellular glutathione homeostasis with 2,5-dihydroxybenzoic acid. *FEMS Microbiology Letters*, *270*(2), 284-290. https://doi.org/10.1111/j.1574-6968.2007.00682.x

Klix, M.B., Verreet, J.A., & Beyer, M. (2007). Comparison of the declining triazole sensitivity of Gibberella zeae and increased sensitivity achieved by advances in triazole fungicide development. *Crop Protection*, *26*(4), 683-690. https://doi.org/10.1016/j.cropro.2006.06.006

Klosowski, A.C., Castellar, C., Stammler, G., & May De Mio, L.L. (2018). Fungicide sensitivity and monocyclic parameters related to the *Phakopsora pachyrhizi*–soybean pathosystem from organic and conventional soybean production systems. *Plant Pathology*, *67*(8), 1697-1705. https://doi.org/10.1111/ppa.12883

Köller, W., Wilcox, W.F., Barnard, J., Jones, A.L., & Braun, P.G. (1997). Detection and quantification of resistance of *Venturia inaequalis* populations to sterol demethylation inhibitors. *Phytopathology*, *87*(2), 184-190. https://doi.org/10.1094/PHYTO.1997.87.2.184

Kotowicz, N.K., Frac, M., & Lipiec, J. (2014). The importance of *Fusarium* fungi in wheat cultivation – pathogenicity and mycotoxins production: a review. *Journal of Animal and Plant Sciences*, *21*(2), 3326-3343.

Laboratoire de la Santé des Végétaux (LSV). (2008). Detection et identification des espèces de *Fusarium* spp. et *Microdochium nival*e sur grains de céréales par isolement mycologique semi sélectif et étude microbiologique. Ref. MH-03.16: version B. (LSV) - station de Malzéville, France, pp 1-25. (In French)

Leroux, P., Albertini, C., Gautier, A., Gredt, M., & Walker, A.S. (2007). Mutations in the CYP51 gene correlated with changes in sensitivity to sterol 14α-demethylation inhibitors in field isolates of *Mycosphaerella graminicola*. *Pest Management Science, 63*(7), 688-698. https://doi.org/10.1002/ps.1390

Leslie, J.F., & Summerell, B.A. (2006). *The Fusarium laboratory manual* (p. 388). Ames, USA: Blackwell Publishing.

Li, X., Sheng, L., Sbodio, A.O., Zhang, Z., Sun, G., Blanco-Ulate, B., & Wang, L. (2022). Photodynamic control of fungicide-resistant *Penicillium digitatum* by vitamin

K3 water-soluble analogue. *Food Control*, 135, 108807. https://doi.org/10.1016/j.foodcont.2021.108807

Liddell, C.M. (2003). Systematics of *Fusarium* species and allies associated with Fusarium head blight. **In:** K.J. Leonard and W.R. Bushnell, *Fusarium head blight of wheat and barley* (pp 35-43). St. Paul, MN, USA: American Phytopathological Society (APS Press).

Lu, X.H., Davis, R.M., Livingston, S., Nunez, J., & Hao, J.J. (2012). Fungicide sensitivity of *Pythium* spp. associated with cavity spot of carrot in California and Michigan. *Plant Disease*, *96*(3), 384-388. http://dx.doi.org/10.1094/ PDIS-07-11-0562

Ma, Z., & Michailides, T.J. (2005). Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. *Crop Protection*, *24*(10), 853-863. https://doi.org/10.1016/j.cropro.2005.01.011

Malbrán, I., Mourelos, C.A., Pardi, M., Oufensou, S., Balmas, V., Delogu, G. ... Girotti, J.R. (2020). Commercially available natural inhibitors of trichothecene production in *Fusarium graminearum*: A strategy to manage *Fusarium* head blight of wheat. *Crop Protection*, *138*, 105313. https://doi.org/10.1016/j.cropro.2020.105313

Masiello, M., Somma, S., Ghionna, V., Logrieco, A.F., & Moretti, A. (2019). In vitro and in field response of different fungicides against *Aspergillus flavus* and *Fusarium* species causing ear rot disease of maize. *Toxins*, *11*(1), 11. https://doi.org/10.3390/toxins11010011

Mateo, E.M., Valle-Algarra, F.M., Jimenez, M., & Magan, N. (2013). Impact of three sterol-biosynthesis inhibitors on growth of *Fusarium langsethiae* and on T-2 and HT-2 toxin production in oat grain under different ecological conditions. *Food Control, 34*(2), 521-529. https://doi.org/10.1016/j.foodcont.2013.05.011

Mateo, E.M., Valle-Algarra, F.M., Mateo, R., Jiménez, M., & Magan, N. (2011). Effect of fenpropimorph, prochloraz and tebuconazole on growth and production of T-2 and HT-2 toxins by *Fusarium langsethiae* in oat-based médium. *International Journal of Food Microbiology, 151*(3), 289-298. https://doi.org/10.1016/j.ijfoodmicro.2011.09.017

Mavroeidi, V.I., & Shaw, M.W. (2005). Sensitivity distributions and cross-resistance patterns of *Mycosphaerella graminicola* to fluquinconazole, prochloraz and azoxystrobin over a period of 9 years. *Crop Protection*, *24*(3), 259-266. https://doi.org/10.1016/j.cropro.2004.07.014

Nakajima, T. (2010). Fungicides application against Fusarium head blight in wheat and barley for ensuring food safety. In O. Carisse, *Fungicides* (pp 140-156). London, UK: Intech Open.. https://doi.org/10.5772/13680

Nene, Y.L., & Thapliyal, P.N. (1993). *Fungicides in plant disease control*. New Delhi, India: Oxford and IBH Publication Company.

Nielsen, L.K., Jensen, J.D., Nielsen, G.C., Jensen, J.E., Spliid, N.H., Thomsen, I.K. ... Jørgensen, L.N. (2011). *Fusarium* head blight of cereals in Denmark: species complex and related mycotoxins. *Phytopathology*, *101*(8), 960-969. https://doi.org/10.1094/PHYTO-07-10-0188

Ochiai, N., Fujimura, M., Oshima, M., Motoyama, T., Ichiishi, A., Yamada-Okabe, H., & Yamaguchi, I. (2002). Effects of iprodione and fludioxonil on glycerol synthesis and hyphal development in *Candida albicans*. *Bioscience*, *Biotechnology*, and *Biochemistry, 66*(10), 2209-2215. https://doi.org/10.1271/bbb.66.2209

O'Donnell, K., Kistler, H.C., Tacke, B.K., & Casper, H.H. (2000). Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. *Proceedings of the National Academy of Sciences of the* U.S.A, *97*(14), 7905-7910. https://doi.org/10.1073/pnas.130193297

O'Donnell, K., Ward, T.J., Geiser, D.M., Kistler, H.C., & Aoki, T. (2004). Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. *Fungal Genetics* and *Biology, 41*(6), 600-623. https://doi.org/10.1016/j.fgb.2004.03.003

Paul, P.A., Lipps, P.E., Hershman, D.E., McMullen, M.P., Draper, M.A., & Madden, L.V. (2008). Efficacy of triazole-based fungicides for *Fusarium* head blight and deoxynivalenol control in wheat: a multivariate meta-analysis. *Phytopathology*, *98*(9), 999-1011. https://doi.org/10.1094/PHYTO-98-9-0999

Paul, P.A., McMullen, M.P., Hershman, D.E., & Madden, L.V. (2010). Meta-analysis of the effects of triazole-based fungicides on wheat yield and test weight as influenced by *Fusarium* head blight intensity. *Phytopathology*, *100*(2), 160-171. https://doi.org/10.1094/PHYTO-100-2-0160

Pereira, C.B., Ward, T.J., Del Ponte, E.M., Mara Moreira, G., Busman, M., McCormick, S.P. ... Tessmann, D.J. (2021). Five-year survey uncovers extensive diversity and temporal fluctuations among Fusarium head blight pathogens of wheat and barley in Brazil. *Plant Pathology*, *70*(2), 426-435. https://doi.org/10.1111/ppa.13289

Petronaitis, T., Simpfendorfer, S. & Hüberli, D. (2021). Importance of *Fusarium* spp. in wheat to food security: A global perspective. In P.R. Strange, L. Korsten & M. Lodovica Gullino (Eds.), *Plant diseases and food security in the 21st century* (pp 127-159). Cham, Switzerland: Springer. https://doi.org/10.1007/978-3-030-57899-2_7

Proctor, R.H., McCormick, S.P., Alexander, N.J., & Desjardins, A.E. (2009). Evidence that a secondary metabolic biosynthetic gene cluster has grown by gene relocation during evolution of the filamentous fungus *Fusarium*. *Molecular Microbiology, 74*(5), 1128-1142. https://doi.org/10.1111/j.1365 2958.2009. 06927.x

Ramirez, M.L., Chulze, S., & Magan, N. (2004). Impact of environmental factors and fungicides on growth and deoxinivalenol production by *Fusarium graminearum* isolates from Argentinian wheat. *Crop Protection*, *23*(2), 117-125. https://doi.org/10.1016/j.cropro.2003.07.005

Rojas, E.C., Jensen, B., Jørgensen, H.J.L., Latz, M.A.C, Esteban, P., Ding, Y., & Collinge, D.B. (2020). Selection of fungal endophytes with biocontrol potential against *Fusarium* head blight in wheat. *Biological Control*, *144*, 104222. https://doi.org/10.1016/j.biocontrol.2020.104222

Rosslenbroich, H.J., & Stuebler, D. (2000). *Botrytis cinerea*-history of chemical control and novel fungicides for its management. *Crop Protection*, *19*(8–10), 557-561. https://doi.org/10.1016/S0261-2194(00)00072-7

Sharifi, K., Zare, R., Zamanizadeh, H.R., Mirabolfathy, M., & Rezaee, S. (2016). Identification of Fusarium species associated with Fusarium head blight of wheat in the North of Iran and phylogenetic analysis of the dominant species. *Rostaniha*, *17*(2), 173-187. https://doi.org/10.22092/botany.2017.109430

Shcherbakova, L., Odintsova, T., Pasechnik, T., Arslanova, L., Smetanina, T., Kartashov, M,, & Dzhavakhiya, V. (2020). Fragments of a wheat hevein-like antimicrobial peptide augment the inhibitory effect of a triazole fungicide on spore germination of *Fusarium oxysporum* and *Alternaria solani*. *Antibiotics*, *9*(12), 870. https://doi.org/10.3390/antibiotics9120870

Shikur Gebremariam, E., Sharma-Poudyal, D., Paulitz, T.C., Erginbas-Orakci, G., Karakaya, A., & Dababat, A.A. (2018). Identity and pathogenicity of *Fusarium* species associated with crown rot on wheat (*Triticum spp*.) in Turkey. *European Journal of Plant Pathology*, *150*(2), 387-399. https://doi.org/10.1007/s10658-017-1285-7

Stehmann, C., & De Waard, M.A. (1996). Sensitivity of populations of *Botrytis cinerea* to triazoles, benomyl and vinclozolin. *European Journal* of *Plant Pathology, 102*, 171-180. https://doi.org/10.1007/BF01877104

Su, Y.Y., Qi, Y.L., & Cai, L. (2012). Induction of sporulation in plant pathogenic fungi. *Mycology*, 3(3), 195-200. Doi: 10.1080/21501203.2012.719042

Suty-Heinze, A., & Dutzmann, S. (2004). Fusarium head blight: an additional strength of prothioconazole. *Pflanzenschutz-Nachrichten Bayer*, *57*(2), 265-282.

Tittlemeier, S.A., Roscoe, M., Trelka, R., Gaba, D., Chan, J.M., Patrick, S.K. ... Gräfenhan, T. (2013). *Fusarium* damage in small cereal grains from Western Canada. 2. Occurrence of *Fusarium* toxins and their source organisms in durum wheat harvested in 2010. *Journal of Agricultural and Food Chemistry*, *61*(23), 5438-5448. https://doi.org/10.1021/jf400652e

Ueda, A., Nishimoto, H., Kato, N., Hirano, T., & Fukaya, M. (2007). Lineages and trichothecene mycotoxin types of *Fusarium* head blight pathogens of wheat and barley in Tokai district. *Research Bulletin of the Aichi-ken Agricultural Research Center*, *39*, 17-23.

Vatankhah, M., Saberi-Riseh, R., Moradzadeh Eskandari, M., & Afzali, H. (2019). Evaluation of some fungicides for the control of Fusarium dry rot of potato. *Journal of Crop Protection*, *8*(3), 275-285. http://jcp.modares.ac.ir/article-3-23405-en.html

Wegulo, S.N., Baenziger, P.S., Hernandez Nopsa, J., Bockus, W.W., & Hallen-Adams, H. (2015). Management of Fusarium head blight of wheat and barley. *Crop Protection, 73*, 100-107. https://doi.org/10.1016/j.cropro.2015.02.025

White, T.J., Bruns, T.D., Lee, S.B., & Taylor, J.W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Shinsky, J.J., & White, T.J. (Eds)., *PCR Protocols: A guide to methods and applications* (pp 315-322). New York, USA: Academic Press. http://dx.doi.org/10.1016/b978-0-12-372180-8.50042-1

Willyerd, K.T., Li C., Madden, L.V., Bradley, C.A., Bergstrom, G.C.,Sweets, L.E. .... Paul, P.A. (2012). Efficacy and stability of integrating fungicide and cultivar resistance to manage *Fusarium* head blight and deoxynivalenol in wheat. *Plant Disease*, *96*(7), 957-967. http://dx.doi.org/10.1094/ PDIS-09-11-0763

Wong, F.P., & Midland, S.L. (2007). Sensitivity distributions of California populations of *Colletotrichum cereale* to the DMI fungicides propiconazole, myclobutanil, tebuconazole, and triadimefon. *Plant Disease*, *91*(12), 1547-1555. https://doi.org/10.1094/ PDIS-91-12-1547

Yang, C., Hamel, C., Vujanovic, V., & Gan, Y. (2011). Fungicides: modes of action and possible impact on nontarget microorganisms. *International Scholarly Research Notices ISRN Ecology,* 1-8. https://doi.org/10.5402/2011/130289

Yin, Y., Liu, X., Li, B., & Ma, Z. (2009). Characterization of sterol demethylation inhibitor-resistant isolates of *Fusarium asiaticum* and *F. graminearum* collected from wheat in China. *Phytopathology*, *99*(5), 487-497. https://doi.org/10.1094/ PHYTO-99-5-0487

Zhang, H., Van der Lee, T., Waalwijk, C., Chen, W.Q., Xu, J., Xu, J.S. ... Feng, J. (2012). Population analysis of the *Fusarium graminearum* species complex from wheat in China show a shift to more aggressive isolates. *PLoS One*, *7*(2), e31722. https://doi.org/10.1371/journal.pone.0031722

Vrste roda *Fusarium* prouzrokovači fuzarioze klasa pšenice u Alžiru: karakterizacija i delovanje triazolnih fungicida

REZIME

Fuzarioza klasa pšenice je važna bolest durum pšenice koja zahteva primenu nekoliko tretmana semena fungicidom kako bi se na zadovoljavajući način suzbila bolest. Istraživanje je sprovedeno kako bi se u uslovima *in vitro* procenila efikasnost komercijalno dostupnih fungicida protiv 18 izolata *Fusarium* spp. koji su sakupljeni na različitim poljima u severo-istočnom delu Alžira. Morfološkom i molekularnom karakterizacijom, na semenu pšenice otkriveno je prisustvo glavnih kompleksa vrsta *F. acuminatum, F. equiseti, F. avenaceum, F. solani, F. culomorum, F. incarnatum-equiseti*, kao i kompleksi vrsta *F. tricinctum* i *F. chlamydosporum*. Antifungalno delovanje fungicida pokazuje da su svi testirani triazoli dokazali efektivnost u inhibiciji porasta micelija različitih testiranih sojeva roda *Fusarium*. Ipak, njihova osetljivost je značajno ($p<0.05$) varirala u zavisnosti od doze i dužine izlaganja pojedinačnim fungicidima. Rezultati su pokazali da su tebukonazol (Raxil i Tébuzole) i kombinacija fludioksonil + difenokonazol u velikoj meri smanjili porast micelija izolata iz roda *Fusarium,* i to respektivno 84.31%, 82.94% i 81.33%, u poredjenju sa samostalnom primenom difenokonazola (73.16%) u preporučenoj dozi nakon pet dana izlaganja. Što se tiče delovanja na klijanje konidija, tebukonazol je bio efikasniji od kombinacije fludioksonil + difenokonazol, koja je uzrokovala deformacije strukture ćelijskog zida i fragmentaciju konidija. Rezultati su dali korisne informacije kao osnovu za odabir pogodnih fungicida za semenski tretman i suzbijanje fuzarioze klasa kao bolesti pšenice.

Keywords: pšenica, *Fusarium*, fungicidi, toksičnost