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First report of *Fusarium asiaticum* and *Fusarium incarnatum* in Algeria, and evaluation of their pathogenicity on wheat crown rot

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Abstract

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Fusarium crown rot (FCR), is one of the most serious wheat diseases. In this study one hundred and seventeen *Fusarium* symptomatic samples were collected in the North-east of Algeria. The obtained isolates (34), were identified on the basis of the morphological and the molecular data using phylogenetics. Accession numbers MW366557 and MW448396, were assigned by NCBI GenBank to isolate FE6 and FE8, identified as *Fusarium asiaticum* and *F. incarnatum*, respectively. A pathogenicity test was conducted on seven bread wheat cultivars (cv) to test their ability to induce FCR by the disease index (DS) parameter. The impact on coleoptile length and weight was estimated by the reduction parameters (RCL and RCW%). The results showed that the strains were saprophytic rather than pathogenic with negligible DS. A statistically significant decrease in coleoptile weight was recorded by FE6 ($P = 0.03 < 0.05$), and FE8 ($P = 0.017 < 0.05$). The cv. Medracen was mostly affected by this reduction with RCW% (11.90 and 35.96%). To the best of our knowledge, for the first time this work confirmed the existence of *F. asiaticum* and *F. incarnatum* species in Algeria.

Keywords: Fungus; *Fusarium asiaticum*; *Fusarium incarnatum*; pathogenicity; Wheat
Abbreviations: FCR*:* *Fusarium* Crown Rot; RCL*:* Reduction Coleoptile Length; RCW*:* Reduction Coleoptile Weight; ITS*:* internal transcribed spacer regions; DS*:* Disease Severity; cv*:* Cultivar

Introduction

Fusarium crown rot (FCR), is one of the most serious cereal crop disease. It is caused by several *Fusarium* genus species. The pathogen colonizes the culm first, then moves into the bundles vascular system, xylem and phloem of the lower internodes, blocking water transmission, nutrient movement and killing the plant (Knight et al., 2017). This anamorphic genus is known to be harmful to numerous plants, including wheat and barley. Furthermore, it has a significant potential to reduce grain productivity and quality (Matny, 2015). For this reason, many studies in Algeria using phytochemical constituents are trying to find an effective solution against these pathogens as alternatives to chemical pesticides (Bendjedid et al., 2022; Benouchenne et al., 2022).

FCR has limited the wheat production around the world (Yekkour et al., 2015). *F. culmorum, F. pseudograminearum, F. verticillioides* and *F. avenaceum* are the FCR associated

species re-ported in Algeria to date (Abdallah-Nekache et al., 2019; Bouanaka et al., 2021b). Additionally, the first reports have been made for *F. cerealis, F. Brachygibbosum F. equiseti*, and *F. chlamydosporum* producing damping-off and crown rot in durum wheat (Bouanaka et al., 2022, 2023a; Bencheikh et al., 2020a, b). This does not rule out the occurrence of other species like *F. asiaticum* and *F. acuminatum* that have not yet been documented in Algeria.

One of the pathogens producing *Fusarium* head blight (FHB), *Fusarium asiaticum*, the sixth lineage of the *F. graminearum* species complex, infects maize (*Zea mays* L.) and other small-seeded cereals, leading to major damages in grain quality and yield around the globe (Goswami & Kistler, 2004).

F. asiaticum is the main causal agent of *Fusarium* head blight in Asia (China, Korea, Nepal, India and Japan) (Van Der Lee et al., 2015; Chen et al., 2020; Tomioka et al., 2020). It was reported that *F. asiaticum* was the species that caused soybean seed rot in Sichuan, China (Chang et al., 2020). *F. asiaticum* is particularly transmissible through crop debris; wheat and rice straw, maize stalks, and from other crops debris. It was exclusively associated with rice-growing areas (Kuhnem et al., 2016).

F. incarnatum also known as *F. semitectum* (Leslie & Summerell, 2006) was the fungus responsible for root rot on peony (Zhang et al., 2020), and the wilting of the crossandra (Mallaiah & Muthamilan, 2015). It has been reported to cause walnut canker, corky dry rot of melons and bananas and other fruits (Seta et al., 2004; McGovern 1994; Marin et al., 1996). It was one of the dominant fungi on pearl millet grain, bell pepper fruit rot in Pakistan, and cucumber fruit rot in Mexico (Wilson, 2002; Tariq et al., 2018; Garcia-Estrada et al., 2020). Although, there are many studies on the involvement of *F. incarnatum* in various diseases, it is often not considered as an important plant pathogen.

The objectives of this study were the isolation of *F. asiaticum* and *F. incarnatum*, isolates FE6 and FE8 have not been previously described in the Algerian territory especially for wheat, the identification of morphological and molecular parameters with a phylogenetic study, as well as the assessement of the ability of FE6 and FE8 isolates to induce FCR and their influence on the wheat vegetative system.

Materials and Methods

Fungal Material

Isolation

During the agricultural season (2018-2019), 117 wheat symptomatic samples or suspected of being symptomatic of *Fusarium* disease were collected in several provinces of Northeastern Algeria, including Constantine, Mila, Guelma, and Oum el Bouaghi. Infected crowns were cut into 10 mm fragments, and the seeds were peeled from their glumes and disinfected in 2 percent sodium hypochlorite (NaClO) for 5 min before being rinsed in three successive baths of sterile distilled water (Benhamou & Chet, 1996). Seeds were plated on potato sucrose agar (PSA) media in sterile Petri dishes (5 fragments/dish) and incubated at 28°C for 7 days after drying between two sheets of sterile paper towels. Fungal colonies suspected of being *Fusarium* were transfered onto PSA media. All isolates were purified using the single-spore technique and kept at -80°C in Eppendorf tubes containing 20% glycerol water.

Macro and Microscopic Characterization

On PSA medium, all the isolates were subcultured. The colony's growth, texture, and appearance were all examined on a macroscopic level, as well as the colour of the Petri dish's face and back. A mycelial disc subculture from the pure culture was plated on Carnation Leaf-Piece Agar (CLA), and Spezieller Nährstoffarmer Agar (SNA) medium in addition to the PSA medium, to examine microscopic features (Leslie & Summerell, 2006). The culture was examined under a light microscope (LEICA DM4000 B LED) after being incubated at 28°C for 10 days.

Micro-conidia, meso-conidia, and mac-roconidia, as well as their shapes and sizes (if present), furthermore, the diagnostic features including, the presence or absence of chlamydospores, micro-conidial chains, and sporodochia were used to identify a species (Leslie & Summerell, 2006).

Molecular Identification and Phylogenetic Analysis

To support these *Fusarium* spp. identifications that were made, based on microscopic and macroscopic evidence. The molecular identification (CEDEX-France) was carried out. Nucleo Spin Plant II, a commercial kit, was used to perform the DNA extraction (Macherey-Nagel Germany), from the mycelium harvested by scraping the surface of the pure isolate culture in a Petri dish. The nucleic acids were isolated using the microwave mini-preparation method outlined by Goodwin & Lee (1993) by adding 100 µL of lysis buffer (50 mM Tris-HCl pH 7.5, 50 mM EDTA, 3% SDS and 1% 2-mercaptoethanol). The final DNA pellet was made in TE 100 µL buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA), and it was kept at -20°C until the use.

Internal transcribed spacer (ITS) regions of rDNA were amplified using a fungal-specific primer ITS-1 (5' CTT GGT CAT TTA GAG GAA GTA A '3) (Gardes & Bruns, 1993). All amplification procedures were completed in a reaction volume of 25 µL that contained 14.1 µL of ultra-pure water

as the sample volume, 1.5 µL MgCl2, 0.2 µL dNTP,1 µL of each pertinent oligonucleotide primer (F and R primers), 0.2 µL of Taq polymerase Promega, and 2 µL of genomic DNA were included in the mixture of 5 µL of Taq Promega buffer. After the deposit of 10 µL of the sample in 1.5% agarose gel electrophoresis, the amplification products were made visible. Staining in an ethidium bromide bath (0.5 µg/mL) was performed after the migration. The Biorad Gel Doc device was then used to view and capture the DNA under UV light (USA).

- PCR temperature condition; Initial denaturation at 95°C for 5 min was followed by 35 cycles of denaturation at 95°C for 30 s, hybridization at 55°C for 30 s, and elongation at 72°C for 45 s before being stored at 10°C until use.
- Purification of PCR products; The NucleoSpin® Gel and PCR Clean-up kits from Macherey-Nagel (Germany) were used to purify the PCR products in accordance with the supplier's recommended technique.
- Sequencing of PCR products; using the Applied Biosystems Big Dye v3.1 kit and the PCR primers used for the amplification of the target fragments, the extracted and purified PCR products were sequenced using the Sanger method (Sanger et al., 1977). Using the program Snap Gene® Viewer 5.2, the acquired sequences were examined and cleaned. The NCBI software BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi Blast) was then used to compare the final sequences with those in the GeneBank database in order to identify the examined isolates based on their percentage of homology with the reference strains.

The MEGA-X software's Clustal W function was used to align multiple sequences in MEGA format utilizing the ITS region sequences of the generated isolates. The Neighbour-Joining (NJ) dendrogram from Saitou & Nei (1987) was implemented in the MEGA software, which was also used to create the phylogenetic tree and distance matrix. The Kimura 2-parameter (K2P) model served as the foundation for the phylogenetic distance (Kimura, 1980).

Plant Material

The coleoptile length, weight and the capacity to produce FCR of seedlings for each isolate (FE6 and FE8) were examined. For this purpose, seven local bread wheat cultivars, including those most frequently produced in the Northeastern part of Algeria (cv. Medracen, El Hachimia, Boumerzoug, Akhamokh, Nif Encer, Arz, and Hidhab) (Bouanaka et al., 2023b) were subjected to an *in vivo* aggressiveness test. The National Institute of Agronomic Research of Algeria (INRAA) provided these cultivars.

FCR Pathogenicity Test

The procedure, used for this test was developed by Bouanaka et al. (2021a). Four cm of cotton was placed inside of sixty-three assay tubes (14 cm x 3 cm). Each tube received 20 mL of distilled water and was sealed with aluminum foil and autoclaved for 2 h at 180°C to sanitize it. A total of 315 seeds (45 seeds/cultivar) had their surfaces cleaned for five minutes with NaClO at 2 % and then three times with sterile distilled water. In each assay tube, five seeds were put on a damp cotton swab. When the seedlings reached the one leaf stage (Zadoks' GS 11) (Zadoks et al., 1974), the tubes were sealed with transparent covers and kept in the host at 28°C for 4 days. Then, the inoculation was carried out as following:

Three control tubes for each cultivar (13 mm disk) were used, three FE6 inoculated tubes, and three FE8-inoculated tubes.

Following inoculation, tubes were placed and distributed randomly into the culture room for 10 days (25/19°C day/night, 16/8 h light/dark cycle), Seedlings were carefully taken from the cotton, when they reached the two-leaf stage (Zadoks' GS 12). On a scale from 0 to 4, FCR severity classifications for crown discoloration were determined in the laboratory:

Class 0 = healthy crown; 1 = light crown browning; 2 = half-crown browning; 3 = complete crown browning; and 4 = seedling death

The McKinney (1923) index, which reflects the proportion of disease severity (i.e., 100), was used to assess the disease severity (DS) of inoculation tubes according to the formula:

$$DS = \{\sum((c \times f)/(n \times N)\} \times 100,$$

where c = disease class, f = frequency, n = number of observations, and N = the highest value of the empirical scale adopted (class 4).

The coleoptiles' length and weight reduction as percentages were determined as follows:

RCL = 100-(Infected CL × 100/Control CL)

RCW = 100-(Infected CW × 100/Control CW)

The test was performed twice back-to-back. Koch's postulates were satisfied by re-isolation of FE6 or FE8 from brown lesions on the crowns.

Statistical Analysis

SPSS software (IBM SPSS Statistics version 25) was used to analyze the data, graphs, tables, and descriptive statistics (mean, standard deviation), comparisons between groups (inoculated FE6, inoculated FE8 and control) were determined by Paired-Samples T-Test. Means of the different treatments were compared using Tukey's honestly significant difference

test. Results were considered significant at the 5% level ($P \leq 0.05$) and highly significant at the 1% level ($P \leq 0.01$).

Results

Morphological Characteristics

FE6; The culture developed in 10 days on PSA medium with abundant, fuzzy, cottony, white to light pink mycelium around the center of the cultures (Figure 1a). The colour of the reverse side was similar to that of the face (Figure 1b). Rather slow development that does not cover a 9 cm Petri dish in 10 days (Figure 1a). FE6 produced abundant sporodochia, which aggregated together in a conspicuous spore mass in the culture's center. Robust, thick-walled macro-conidia were intermediate in length (Figure 1f). Micro-conidia and meso-conidia were produced on PSA (Figure 1c). Macro-conidia were absent. On SNA medium at 28°C for 10 days, macro-conidia were robust, thick-walled, apical and basal, curved cells, usually with 3 to 4 septa (Figure 1f). On CLA medium, abundant chlamydospores formed on pieces of carnation leaves, but also formed directly on the agar (Figure 1d).

For FE8 isolates, the results disclosed that, the 10-day-old culture on PSA medium, it had an abundant, fuzzy, aerial, cottony, and snow-white to light yellow mycelium that was denser around the culture's center (Figure 2a). The reverse side was even more yellow to orange towards the center (Figure 2b). With fairly fast development, the 9 cm Petri dish was covered in 10 days (Figure 2a). FE8 produced abundant micro-conidia and meso-conidia, which aggregated together in a well visible spore mass in the PSA culture's center (Figure 2c). Macro-conidia had from 2 to 5 septa on SNA medium (Figure 2d). On CLA medium at 28°C for 10 days, macro-conidia were robust, thick-walled cells. Round chlamydospores had robust walls (Figure 2e, f).

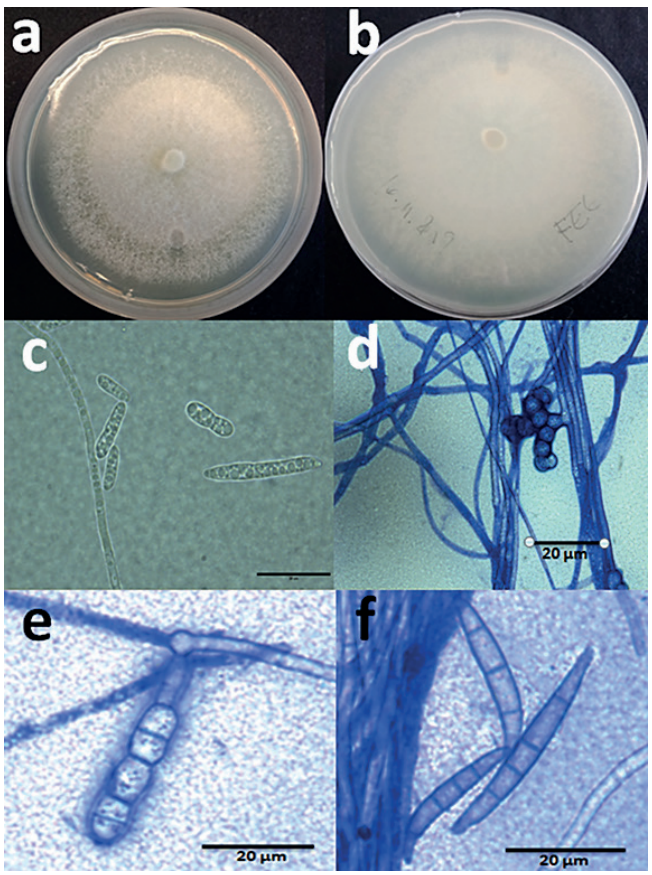

Fig. 1. Macroscopic appearance of *F. asiaticum* (FE6) after 10 days of incubation on PSA medium, a: the face and b: the reverse. Different *F. asiaticum* conidia aspects (FE6); c: micro and meso-conidia on PSA, d: chlamydospores on CLA. e, f: chlamydospores and macroconidia on SNA. Scale bar 20 µm

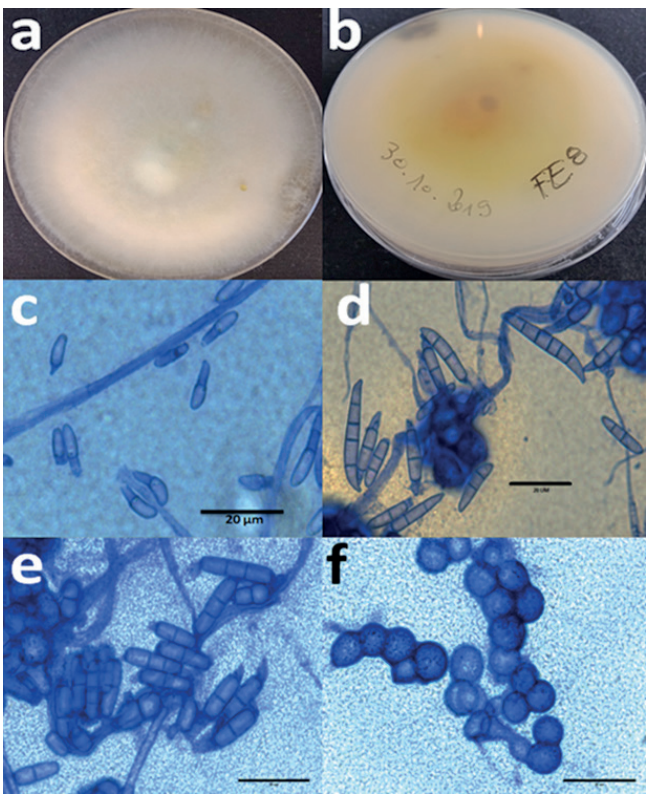

Fig. 2. Macroscopic appearance of *F. incarnatum* (FE8) after 10 days of incubation on PSA medium; a: the face and b: the reverse. Different *F. incarnatum* conidia aspects (FE8); c: micro-conidia and meso-conidia on PDA, d: macro-conidia on SNA and e, f: macro-conidia and chlamydospores on CLA. Scale bar 20 µm

Molecular Identification and Phylogenetic Analysis

Identification for FE8 was assessed on the basis of macroscopic data and microscopic features (Leslie & Summerell, 2006) and confirmed by molecular identification. The ITS1-2 sequence of each isolate was compared to reference sequences collected from GenBank (Tables 1 and 2). Isolate FE6 was identified as *F. asiaticum*. Isolate FE8 was identified as *F. incarnatum* and the accession numbers (MW366557 and MW448396) were assigned by NCBI GenBank, respectively.

Table 1. The comparison of the DNA sequence of FE6 isolate (obtained with ITS) to the referred sequences collected from the Genbank (NCBI)

Description	Query Length	Total score	Query cover	Per. ident	Host	Country	Accession
F. asiaticum strain FE6 internal transcribed spacer 1	484 bp				Wheat	Algeria	MW366557
F. asiaticum isolate 97b internal transcribed spacer 1	495 bp	889	100%	99.79%	Not mentioned	China	KY466889
F. asiaticum isolate 90b internal transcribed spacer 1	494 bp	889	100%	99.79%	Not mentioned	China	KY466882
F. asiaticum isolate 81b internal transcribed spacer 1	500 bp	889	100%	99.79%	Not mentioned	China	KY466873
F. asiaticum isolate 77b internal transcribed spacer 1	501 bp	889	100%	99.79%	Not mentioned	China	KY466869
F. asiaticum isolate 73b internal transcribed spacer 1	500 bp	889	100%	99.79%	Not mentioned	China	KY466865
F. asiaticum isolate 71b internal transcribed spacer 1	499bp	889	100%	99.79%	Not mentioned	China	KY466863
F. asiaticum isolate 58b internal transcribed spacer 1	499 bp	889	100%	99.79%	Not mentioned	China	KY466850
F. asiaticum isolate 102a internal transcribed spacer 1	520 bp	889	100%	99.79%	Not mentioned	China	KY272847
F. asiaticum isolate 90a internal transcribed spacer 1	518 bp	889	100%	99.79%	Not mentioned	China	KY272836
F. asiaticum isolate 89a internal transcribed spacer 1	517 bp	889	100%	99.79%	Not mentioned	China	KY272835
F. asiaticum isolate 85a internal transcribed spacer 1	521 bp	889	100%	99.79%	Not mentioned	China	KY272831
F. asiaticum isolate 77a internal transcribed spacer 1	516 bp	889	100%	99.79%	Not mentioned	China	KY272824

Table 2. The comparison of the DNA sequence of FE8 isolate (obtained with ITS) to the referred sequences collected from the Genbank (NCBI)

Description	Query Length	Total score	Query cover	Per. ident	Host	Country	Accession
F. incarnatum strain FE8 internal transcribed spacer 1	492 bp				Wheat	Algeria	MW448396
F. incarnatum isolate 15 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1	567 bp	909	100%	100%	Wheat	Jordan	MN534779
F. incarnatum clone 2014_5 internal transcribed spacer 1	528 bp	909	100%	100%	*Cucurbita* root	USA	MN521835
F. incarnatum culture-collection NCCPF:960005 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1	554 bp	909	100%	100%	*Homo sapiens*	India	KM921663
F. incarnatum strain 171867 18S small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1	538 bp	904	100%	99.80%		Mexico	MK174967
F. incarnatum isolate ML1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1	573 bp	904	100%	99.80%	*Toxicodendron sylvestre*	China	MG655148
F. incarnatum strain CBA-5 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1	541 bp	902	100%	99.80%	*Panax ginseng*	China	MT560227
F. incarnatum strain GFR07 internal transcribed spacer 1	528 bp	902	100%	99.80%	*Lycium barbarum* L.	China	MT447512
F. incarnatum strain ZMXR9 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1	544 bp	902	100%	99.80%	*Stipa purpurea*	China	MT446114
F. incarnatum isolate R2 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1	546 bp	902	100%	99.80%	Barber shops in informal settlements	Kenya	MT420619
F. incarnatum clone 2014_32 internal transcribed spacer 1	516 bp	902	100%	99.80%	*Cucurbita* root	USA	MN521857
F. incarnatum isolate ZB11263541 internal transcribed spacer 1	521 bp	902	100%	99.80%	Wolfberry	China	KX783371
F. incarnatum isolate ZB11263542 internal transcribed spacer 1	524 bp	902	100%	99.80%	Wolfberry	China	KX783370

FCR Severity

The crown inoculation test results in growth room evaluated as a percentage of the disease index DS showed that FE6 and FE8 caused FCR to all bread wheat cultivars tested, all values were greater than 0.05. The values recorded were very low and even negligible (Table 3).

The pathogenicity test was performed on 7 bread wheat varieties to determine the impact of these isolates on the wheat basal part (crown).

The DS values were more or less very close varying between 0.05% for cv. Boumerzoug and 0.34% for cv. Hidhab. The RCL and RWL values were positive, except for cv. Boumerzoug where contaminated by FE6 and even FE8 did not influence coleoptile length or weight, with even negative values recorded (-7.64 and -4.96%), respectively (Table 3). There were no statistically significant differences between $DS_{asiaticum}$ and $DS_{incarnatum}$ ($P = 0.88 > 0.05$). It was the same for $CL_{asiaticum}$ and $CL_{incarnatum}$ ($P = 0.35 > 0.05$), and also for $CW_{asiaticum}$ and $CW_{incarnatum}$ ($P = 0.055 > 0.05$). Both strains seemed to have the same pathogenicity.

Discussion

The most severe infections affecting wheat plant roots, stems, spikes, and at all growth stages are caused by the genus *Fusarium*. Studies on *F. culmorum*, the principal agent causing wheat FCR and FHB in Algeria, with an emphasis on its incidence, pathogenicity, and diversity, have been conducted (Abdallah-Nekache et al., 2019; Laraba et al., 2017). There has however been little global research on the pathogenicity of *F. asiaticum* and *F. incarnatum* on wheat, and the other *Fusarium* pathogenic species are still not well understood. Studies that quantify their impacts on coleoptile length and weight, as well as their capacity to trigger FCR in particular are needed.

Following identification, the findings revealed a wide variety of isolated species, as well as the presence of novel species that had never been recorded in Algeria. The FE6 and FE8 isolates were morphologically identified as *F. asiaticum* and *F. incarnatum*. They were isolated from wheat grains in Algeria's Constantine area in the North. Sequencing of the rDNA internal transcribed spacer's ITS1-2 region was used for molecular identification (contained the sequences of internal transcribed spacer 1, 5.8S ribosomal RNA and internal transcribed spacer 2) (Johannesson & Stenlid, 1999). This study offers the first proof of *F. asiaticum* and *F. incarnatum* species in Algerian wheat fields.

Comparison of the ITS1-2 rDNA sequence of FE6 to the reference sequences collected in the Genbank database

Table 3. The disease severity (DS), and the percentage of reduction in coleoptile length and weight (%RCL and %RCW) obtained by the tube test

Parameters	Disease Severity (DS%)		Coleoptile Length (mean±Std. Dev)(mm)			Coleoptile Weight (mean± Std. Dev)(mg)			% Reduction in coleoptiles Length and Weight			
									F. asiaticum		*F. incarnatum*	
Grain Cultivar	DS *F.asiaticum*	DS *F. incarnatum*	Infected CL (mm) *F. asiaticum*	Infected CL (mm) *F. incarnatum*	Control CL (mm)	Infected CW (mg) *F. asiaticum*	Infected CW (mg) *F.incarnatum*	Control CW (mg)	RCL	RCW	RCL	RCW
Medracen	0.18±0.05^{a}	0.14±0.09^{a}	151.66±5.29^{ab}	138.21±15.35^{a}	157.16±4.61^{bc}	90.10±2.55^{ab}	65.50±4.34^{a}	102.28±18.64^{a}	3.49	11.90	12.05	35.96
El Hachimia	0.17±0.04^{a}	0.17±0.05^{a}	146.07±2.24^{ab}	147.00±7.00^{a}	150.45±3.62^{abc}	79.80±4.55^{a}	77.05±9.20^{abc}	85.46±4.20^{a}	2.91	6.62	2.29	9.84
Boumerzoug	0.05±0.05^{a}	0.12±0.01^{a}	160.24±1.92^{b}	156.25±5.22^{a}	148.86±3.53^{abc}	88.38±7.56^{ab}	83.97±3.39^{c}	94.84±4.78^{a}	-7.64	6.81	-4.96	11.46
Akhamokh	0.20±0.06^{ab}	0.08±0.02^{a}	144.13±20.92^{ab}	132.93±3.60^{a}	136.85±6.23^{a}	82.92±5.13^{a}	77.86±2.79^{abc}	85.99±4.77^{a}	-5.31	3.57	2.86	9.45
Nif Encer	0.08±0.04^{a}	0.10±0.04^{a}	127.73±4.08^{a}	144.53±20.49^{a}	144.57±8.98^{ab}	77.49±6.99^{a}	79.72±3.24^{bc}	82.30±1.87^{a}	11.64	5.84	0.02	3.13
Arz	0.10±0.05^{a}	0.26±0.10^{a}	151.06±4.43^{ab}	143.93±3.73^{a}	155.20±4.63^{bc}	76.37±1.96^{a}	69.86±3.98^{ab}	80.96±4.20^{a}	2.66	5.66	7.26	13.71
Hidhab	0.34±0.08^{c}	0.21±0.08^{a}	153.64±11.99^{ab}	144.60±3.44^{a}	163.61±3.09^{c}	103.97±9.55^{b}	86.54±2.69^{c}	106.67±12.75^{a}	6.09	2.53	11.61	18.87

For each column, the values (mean± Std. Deviation) with different letters denote the statistical significance determined by ANOVA followed by Tukey test ($P < 0.05$).
No statistically significant differences between the lengths of infected coleoptiles and the control ($P > 0.05$).
A statistically significant difference was recorded between $CW_{asiaticum}$ and $CW_{control}$ ($P = 0.03 < 0.05$)

(NCBI), showed 100% similarity to *F. asiaticum* strain (isolate 97b) in China with accession number KY466889 (Table 1). The FE8 showed 100% similarity rate with *F. incarnatum* strain (isolate 15) from Jordanian wheat (MN534779) (Table 2). This confirmed the morphological identification results.

Phylogenetic analysis was deduced by neighborhood analysis (NJ) of the ITS region, our isolates were grouped into 3 distinct clades (Figure 3). The third clade groups our strain with three others, all from China (Table 1). The phylogenetic analysis groups FE8 isolate into 2 distinct clades (Figure 4). The second clade includes *F. incarnatum* with isolate ZB11263542 with accession number KX783371, isolated from the Wolfberry plant in China (Table 2).

Pathogenicity and aggressiveness evaluation of FE6 and FE8 on wheat FCR was carried out on 7 bread wheat varieties to determine their FCR ability, as well as their influences on the vegetative system length and weight (Table 3). Although, the resistance/susceptibility level of the tested cultivars varied, the strains *F. asiaticum* and *F. incarnatum* were found to have very low pathogenic with very low DS values (≤ 0.34), or even negligible. They can be considered as non-pathogenic, nevertheless for our strains (Table 3). Contrary to these results many works have reported that *F. asiaticum* is the head blight main causal agent in Asia (Tomioka et al., 2020). *F. incarnatum* was responsible for various plant diseases in the world (Ramdial et al., 2016; Martínez et al., 2020a, b; Martínez-Zamora et al., 2021; Seta et al., 2004). In China, *F. incarnatum* caused black spot disease of jujube (Guo et al., 2016), root of *Morus alba* (Chen et al., 2017).

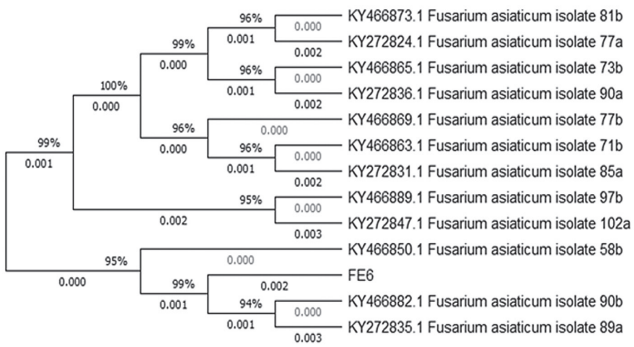

Fig. 3. Phylogenetic relationships of *F. asiaticum* isolates (FE6) inferred by Neighbour-Joining (NJ) analysis of ITS sequences

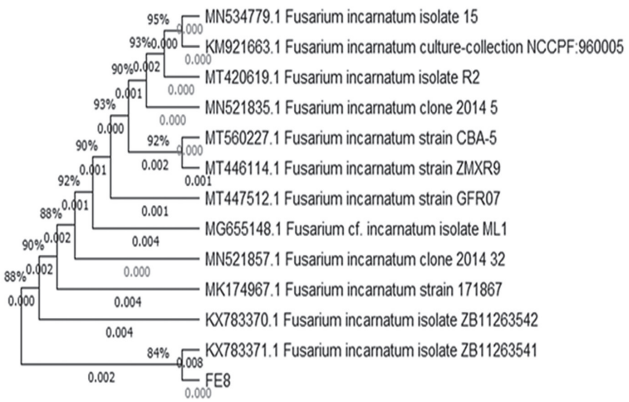

Fig. 4. Phylogenetic relationships of *F. incarnatum* isolates (FE8) inferred by Neighbour-Joining (NJ) analysis of ITS sequences

There were no statistically significant differences between the infected coleoptile lengths and the control ($P > 0.05$). FE6 and FE8 did not have an influence on the coleoptile length compared to control, but a statistically significant difference was recorded between $CW_{asiaticum}$ and $CW_{control}$ ($P = 0.03 < 0.05$). The same was revealed for $CW_{incarnatum}$ with $CW_{control}$ ($P = 0.017 < 0.05$). These results confirmed the CW negative effect. Thus, the FE6 and FE8 aggressiveness was manifested only by a modest reduction in wheat coleoptile weight (%RCW).

Medracen was the most sensitive cultivar and had the highest reduction rates with RCW (%) = 11.90 and 35.96% for FE6 and FE8, respectively (Table 3). The cv. Boumerzoug was the exception with negative RCL% values (-7.64 and -4.96%) towards FE6 and FE8, respectively (Table 3). This study showed the importance of searching for new wheat *Fusarium* species in FCR and FHB infected samples, and the need for further research on other *Fusarium* species that could be a potential wheat crop threat.

Conclusions

This study identified for the first time *F. asiaticum* and *F. incarnatum* in Algerian wheat, and highlighted their influence on seven bread wheat varieties. The severity was very limited on FCR and coleoptile length reduction. However, a negative affect was demonstrated on the vegetative system fresh weight. The prevalence of these pathogens in wheat fields and its effects on crop productivity will have an impact on future scientific research on wheat FCR.

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