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PATHOGENICITY OF *FUSARIUM* AND *MICRODOCHIUM* SPECIES ASSOCIATED WITH CROWN ROT OF BARLEY (*HORDEUM VULGARE* L.)

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Abstract

Description of the subject: Fusarium root and crown rot are a very important complex disease of cereals worldwide which may lead to very high yield losses.
Objective:. The objective of this study is to evaluate the pathogenicity of four *Fusarium* and *Microdochium* species causing crown rot (FCR) in barley under controlled conditions.
Methods: Four fungal species were isolated from barley seedlings. Their pathognenicity was evaluated on the susceptible barley cultivar, as well as its effect on seedling growth and emergence.
Results: All species tested caused crown rot symptoms on the barley seedlings. *F. culmorum* and *F. graminearum* were the most pathogenic species, whereas, *F. equiseti* and *M. nivale* have intermediate pathogenicity. The root and shoot length were significantly reduced in infected seedlings.
Conclusion: Pathogenicity assessment of *Fusarium* isolates is crucial to develop effective control strategies against crown rot disease of barley.
Key words: Barley, *Fusarium spp*., crown rot, seedling emergence, growth parameters.

ÉTUDE DU POUVOIR PATHOGENE DE QUELQUES ESPÈCES DU GENRE *FUSARIUM* ET *MICRODOCHIUM* ASSOCIÉES A LA POURRITURE DU COLLET DE L'ORGE (*HORDEUM VULGARE* L.)

Résumé

Description du sujet : La pourriture racinaire, et la pourriture du collet sont des maladies complexes très importantes des céréales dans le monde entier, qui peuvent entraîner des pertes de rendement très importantes.
Objectifs : L'objectif de ce travail est d'évaluer la pathogénicité de quatre espèces de *Fusarium* et de Microdochium causant la pourriture du collet (FCR) de l'orge dans des conditions contrôlées.
Méthodes : Quatre espèces fongiques ont été isolées à partir des plantules d'orge. Leur pathogénicité a été évaluée sur une variété d'orge sensible, ainsi que leur impact sur l'émergence et la croissance des plantules contaminées.
Résultats : Toutes les espèces testées ont causé des symptômes de pourriture du collet sur les plantules d'orge. *F. culmorum* et *F. graminearum* étaient les espèces les plus pathogènes, tandis que *F. equiseti* et *M. nivale* ont un pouvoir pathogène intermédiaire. La longueur des racines et des tiges ont été considérablement réduite chez les plantules contaminées.
Conclusion : L'évaluation de la pathogénicité des isolats de *Fusarium* est cruciale pour la mise au point de stratégies de lutte efficaces contre la pourriture du collet chez l'orge.
Mots clés: Orge, *Fusarium spp*., pourriture du collet, émergence, paramètres de croissance.

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INTRODUCTION

Barley (*Hordeum vulgare* L.) is an important cereal crop for supplying human and animal food in the world. In Algeria, it is one of the main sectors of agricultural production particularly in semi-arid areas, and it shows an adaptation as compared with other cereals [1]. Despite this economic importance, barley suffers from many phytosanitary problems, which are probably the main causes of low production [2].

The genus *Fusarium* is one of the most relevant fungus affecting cereal crops in many areas of the world. The fungus can be soil borne, seed borne or carried in plant residue and can be recovered from any part of the plant from the deepest root to the highest flower [3]. Many *Fusarium* species are widespread and occur commonly as pathogens of wheat and barley, causing different diseases. Root rot, and crown rot are economically important diseases of cereals in many grain producing areas worldwide and appear to be particularly enhanced by dry climatic conditions [4]. Root colonization negatively affects plant development and leads to systemic plant invasion by tissue-adapted fungal strategies [5]. Some of those pathogens are able to form mycotoxins- secondary metabolites (eg fumonisin, moniliformin, trichothecenes) with possible health hazards and significant influence on food safety [6].

The Fusarium crown rot infection cycle consists of three distinct phases: initial infection in which the pathogen proliferates around the site of infection, a lag phase in which little increase in fungal biomass or symptom development is observed, and a necrotrophic phase in which the pathogen rapidly colonizes internal crown tissue leading to development of necrotic lesions and stem browning [7]. The objective of this study was to compare the relative pathogenicity of *Fusarium* and *Microdochium* isolates on barley seedlings and their impact on seedling growth and emergence.

MATERIALS AND METHODS

1. Presentation of the study area

Our surveys were carried out at a three sites, located in the Mascara department from February to March during the crop season 2017-2018 (Fig. 1). The first site is located between Mascara and Maoussa (35°23′ N 0°11′ E). The second site, El Hashem, is located in the East of Mascara (35°22′ N 0°29′ E). The third site, Ghriss, is located in the South from Mascara (35°14′ N 0°9′ E).

Figure 1: Study area of Mascara, Algeria, indicating the three districts sites covered by the survey

2. Sample collection

Sampling was carried out randomly at the edges and the center of the plots. This sampling, mainly concerned barley plants showing symptoms associated with seedling blight, and rotting of roots, crowns and stems of barley seedlings. Samples collected at each site were immediately placed in Kraft paper bags that record the sampling site, phenological stage of the plant, and the date of collection. Samples were then transported to the laboratory for isolation and identification of phytopathogenic fungi.

3. Isolation and purification of plant pathogenic fungi

The diseased stems collected from each field were washed thoroughly under tap water. Three diseased crown or stem segments (1–1.5 cm) were collected from each plant. These sections were surface-sterilized sequentially with 3% sodium hypochlorite for 1–2min, rinsed thrice with sterile distilled water, and then dried on a sterile filter paper. The samples were placed onto potato dextrose agar (PDA) (200 g of peeled potato, 20 g of dextrose, and 20 g of agar in 1,000 mL distilled water) medium containing 150µg/mL streptomycin. The plates were incubated at 25◦C for 7 days under a day/night photoperiod of 12/12 h.

Resulting fungal colonies were re-isolated on PDA to develop monoxenic cultures by taking hyphal tips. The isolates of *Fusarium spp*. were identified and purified using the single spore isolation protocol described by Xu et *al*. [8].

4. Effects of Fusarium spp isolates on Bartley seedling

4.1. Pathogenicity test

Four isolates of *Fusarium* and *Microdochium* species recovered from barley seedling were used in this study. The following table represents the origin of the isolates tested.

Table 1: The isolates used in this study and their respective regions

Fungal species	Code	Plant part	Site of sampling
Fusarium culmorum	F1	Crown	Ghriss
Fusarium graminarum	F2	Crown	El Hachem
Fusarium equiseti	F3	Root	Maoussa
Microdochium nivale	F4	Root	El Hachem

The plant material used in all experiments is the susceptible barley variety Saida 183. The seeds of this variety were supplied by The Technical Institute of Field Crops in Saida.

Pots were prepared using soil autoclaved at 148 °C at for 45 min. The fungal isolates of *F. culmorum, F. graminearum, F. equiseti* and *M. nivale* were each grown on PDA medium for about 7 days. An agar plug (1 cm diameter) with mycelium only was cut from the periphery of the agar cultures and placed agar-side-up at the bottom of four 4-cm-deep holes made previously in each pot. Barley seeds (*Hordeum vulgare* L), variety Saida 183, surface-sterilized for 1 min in an aqueous solution of 0.6% NaOCl and rinsed twice in sterile deionized water, was then placed on top of the agar plug and covered with soil. An agar plug without fungus was used as a control treatment) [9].

The pots were watered daily with sterilized tap water and grown in a greenhouse at 21 to 25°C. The test included 5 replicates for each isolate. The emergence of the seedlings was evaluated 2 weeks after planting.

The assessment of disease severity index was carried out on each plant within each pot three weeks after inoculation. FCR disease symptoms were assessed using a four-point Browning Index (BI) scale (0: symptom less, 1: slightly necrotic, 2: moderately necrotic, 3: severely necrotic))[7].

Disease index (DI) was then calculated by using the following formula: $DI = \Sigma (Rn\ X/4\ N) \times 100$

Where: Rn is the number of plants in the category, X is the scale value of each plant, and N is the total number of plants assessed [10].

4.2. Disease impacts on plant development

After evaluation of the disease index, the plants are carefully pulled up and washed. Root length (cm), and growth of aerial parts of inoculated and non-inoculated (control) seedlings were assessed in order to determine the effect of infection on seedling development.

5. Statistical analysis

Effects of *Fusarium spp*. isolates on Barley seedling emergence, inhibition of the emergence rate (IIR) and disease index (DI) were expressed by mean percentage values± standard deviation (SD). Data were analyzed by a one-way analysis of variance (ANOVA). Tukey test (Least significant differences, LSD) was used to estimate least significant range between means. Also, impacts of *Fusarium spp*. on Bartley growth traits (stem and root lengths) were analyzed by two-way ANOVA. Flowed, by Dunnett multiple comparisons. All results were statistically compared to their corresponding controls.

RESULTS

1. Isolation and identification of fungi

Crown rot infection caused by *Fusarium* spp. was observed in various regions in Mascara. This Disease causes browning and necrosis of roots and consequently reduces seedling vigour and affects root and crown functions. Disease infects the stem base of barley causing necrosis and dry rot of the crown bases, often extending up 2 at 3 nodes, pinkish fungal growth was formed on lower nodes especially during moist weather (Fig. 2).

Fusarium species associated with symptoms were isolated and identified from different barley crops from Mascara. Identification of isolates recovered from roots and stem base sections of barley samples mainly revealed the presence of *F. culmorum, F. graminearum F. equiseti* and *M. nivale. F. culmorum* and *F. graminearum* dominated the fungi isolated from roots, whereas F. *equiseti and M. nivale* was the predominant *Fusarium* species isolated from crowns.

Figure 2: Symptoms of Fusarium crown rot (FCR) on barley

2. Effect of Fusarium spp on plant emergence

The barley variety Saida 183 is known for its high germination and emergence capacity. The emergence of the uninoculated control barley seedligs under laboratory conditions was 97.67 % (Table 2).

Fusarium spp. isolates had a significant effect on emergence of barley plants. The results obtained showed a variability in the emergence percentages of seedlings according to the pathogen inoculated.

F. culmorum induced highly emergence percentage reduction of barley seedlings 35.66%. The percentage of emerged plants decreased after inoculation with *F. graminearum* by 27%, when compared with uninoculated control, while the lowest percentage of inhibition was recorded in *F. equiseti* and *M. nivale* with 25.33% and 17.66 % respectively.

Table 2: Emergence rate of barley seedlings contaminated by *Fusarium spp*

Fungus	Control	*F. culmorum*	*F. graminarum*	*F. equiseti*	*M. nivale*
ER% ± SD	97.67±2.52	62.00±4.00****	70.67±2.08*****	72.33±4.04****	80.00±5.00%***

ER: Emergence rate ± standart deviation; ****: very highly significant difference; ***: highly significant difference at p=0.05.

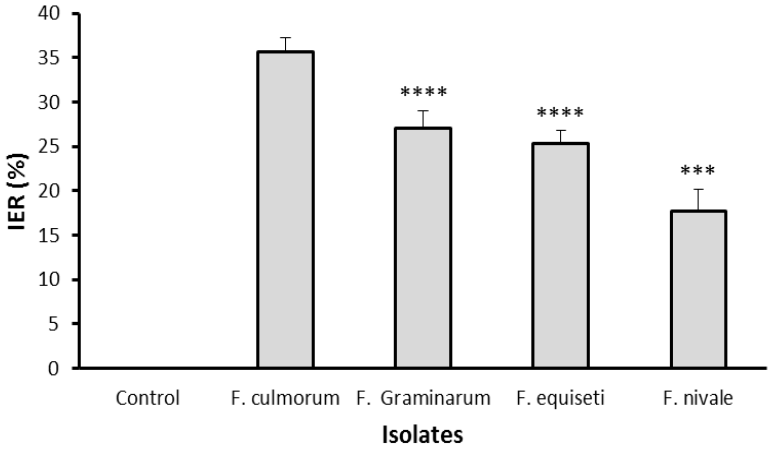

Figure 3: Effect of Fusarium isolates on the inhibition of the emergence of Barley seedlings (IIR: inhibition of the emergence rate). ****: very highly significant difference; ***: highly significant difference at p=0.05.

3. Effect of Fusarium spp on the disease index

Inoculations with Fusarium species caused discoloration of the subcrown internodes and crowns. *F. culmorum* and *F. graminearum* caused the greatest discoloration on both plant parts, whereas *M. nivale* and *F. equiseti* caused the least discoloration.

Diseased crowns are brown in contrast to the white color of healthy crowns (Fig. 4). Barley crowns rot to a greater or lesser degree depending on the stage and severity of disease development. Plants with severe crown damage generally do not survive. Those with moderate root and crown damage tiller sparsely.

Figure 4: Effect of artificial inoculation with *Fusarium spp* on barley seedlings
(C: control, F1: F. *culmorum*; F2: *F. graminearum* ; F3: *F. equiseti*; F4: *M. nivale*)

All isolates of the three *Fusarium spp*. and *M.nivale* were able to cause crown rot of barley seedling, however, there was a significant difference in their pathogenicity.

Fusarium culmorum proved to be the most severe pathogen and had a mean crown rot index of 2.8 (table 3). There was no significant difference in the mean crown rot index between *F. culmorum* and *F. graminarum* (mean = 1.9) ($p < 0.05$), Whereas, *Fusarium equiseti* and *M. nivale* exhibited weak aggressiveness on barley seedlings and had a mean crown rot index of 0.63 and 0.9, respectively. Control plants did not develop any symptoms.

Table 3: Mean disease index on barley seedlings evaluated 21 days after inoculation

Fungal species	Control	*F. culmorum*	*F. graminarum*	*F. equiseti*	*M. nivale*
DI ±SD	0	2.8±0.35****	1.9±0.36****	0.63±0.35*	0.9±0.46**

DI±SD: Disease index ± standard deviation; ****: very highly significant difference; ***: highly significant difference, **: moderate significant difference, *: significant difference at p=0.05.

4. Disease impacts on barley growth parameters

Laboratory infection of barley seedlings by fungi reduced root length, and shoot length of barley seedlings (figure 5). The root length of control barley plant was 17.50 cm, which was reduced to 14.36 and 14.70 cm after infection by F. *graminearum* and F. *culmorum* respectively. The length of shoot system was also affected. Untreated barley plants were 22.30 cm long which became reduced to 17.73 cm after infection by F. *culmorum* and 18.63 cm after infection by *F. graminearum*. There was a significant decrease in the growth of barley shoot in infected plants compared with healthy plants (Fig. 5). On the other hand, the weakest effect on the growth of barley seedlings was recorded for *F. equiseti* and *M. nivale*.

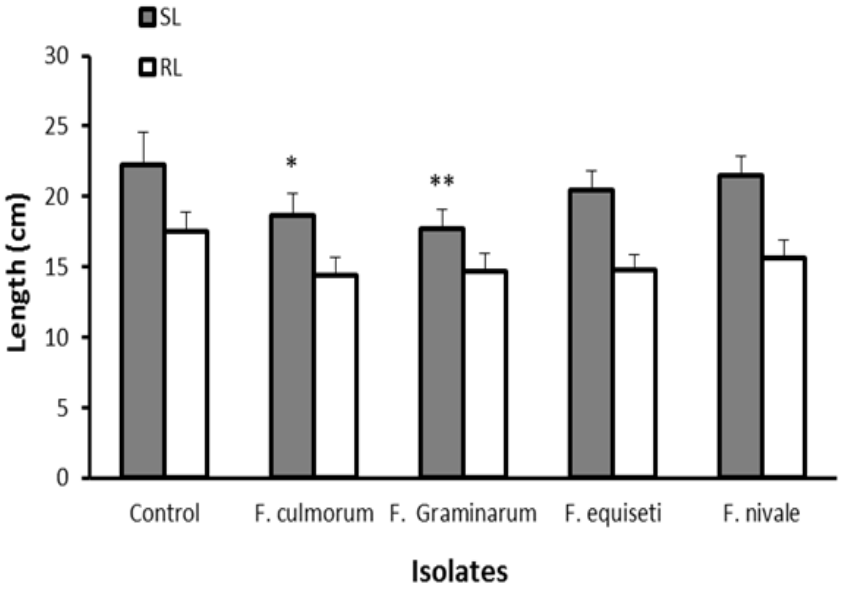

Figure 5: Effect of *Fusarium spp* on barley seedling growth (RL: root length; SL: stem length). **: moderate significant difference, *: significant difference at p=0.05.

DISCUSSION

Fungal pathogens with a necrotrophic phase of their infection cycle, such as *Fusarium spp*., cause substantial losses in grain crops globally, amounting to billions of dollars every year [11]. Crown rot of barley is caused by a complex of species, that includes *Fusarium pseudograminearum*, *F. graminearum*, *F.culmorum*, *F. poae*, *F. equiseti*, *F.avenaceum* and *Microdochium nivale* (formerly known as *Fusarium nivale*) [9, 12, 13]. The infection of the root and crown causes the vascular system to constrict, which limits the uptake and transport of water, causing whiteheads at the filling stage [12, 14].

Four fungal species were collected from the barley fields survey across Mascara city. Morphological identification of isolates revealed the presence of *F. culmorum*, *F. graminearum F. equiseti* and *M. nivale.* Their pathognenicity was evaluated on the susceptible barley cultivar "Saida 183". The mycelium agar plug inoculation technique was used to evaluate the pathogenicity of *Fusarium* and *Microdochium* isolates on barley seedlings. This technique was adopted, since it has proved to be easier and require less time. In addition, some isolates of *F.spp* failed to sporulate abundantly. This technique has also been used by Fernandez and Chen [9] and Tunali *et al.* [15].

The typical necrosis symptoms were present after inoculation with all the Fusarium species, confirming their ability to cause FCR. For all measurements taken, *F. culmorum* and *F. graminearum* induced the highest disease severity index, whereas *F. equiseti* and *M. nivale* were the weakest pathogenic species. Variation in the disease severity can result from differences in the aggressiveness of fungal isolates. Variability among species and isolates should be considered when designing strategies for controlling this disease.

The results of seedling pathogenicity tests are in agreement with previous reports on the fungal pathogenicity of crowns of barley.

Fernandez and Chen [9] found that *F. culmorum* and *F. graminearum* were more aggressive as crown rot pathogens than *F. avenaceum* under greenhouse conditions. Other Fusarium strains that caused less severe FCR on wheat or barley seedlings in greenhouse tests included some isolates of *F. equiseti, F. acuminatum, F. proliferatum* and *M.nivale* [16, 12 ,9].

Both isolates recorded from root or crown of barley seedling induces crown rot in barley seedlings after 21 days of inoculation. These findings are in agreement with Paulitz [17], who found that the symptoms of Fusarium root rot crown rot are often associated with those of crown rot because these pathogens are also able to infect the roots and grow to the crown.

All the inoculated isolates caused emergence percentage reduction of barley seedlings. Fernandez and Chen [9] also found that *F.culmorum* affected plant emergence to a greater extent than *F.graminearum, F. avenaceum, F. equiseti,* or *F. poae.*

In artificial inoculation studies most of the *Fusarium* isolates tested reduced seedling root and shoot growth barley seedlings. Growth of the surviving plants was retarded most by *F. culmorum* and *F. graminearum*. Whereas, *F. equiseti* and *M. nivale* caused the weakest reduction in root length, and shoot length of barley seedlings which was not significantly different from the control. Similar results had been recorded by Neumann and Xue [18], who tested the reactions of field pea cultivars to four races of *Fusarium oxysporum* f. sp. pisi., they found that wilt severity was significantly correlated with reduction in shoot length, and that the reduction in shoot length could be used to supplement the visual severity rating for fusarium wilt in field pea.

CONCLUSION

FCR disease is an endemic disease in barley crops caused by different pathogens of the genera *Fusarium*. Predictions associated with climate change suggest considerable yield loss and widespread distribution of the pathogens associated with this crown and root rot complex in Algeria. Therefore, preventive measures should be taken to reduce pathogen infection and spread, by using integrate pest management for the disease through using resistance cultivars, biological control, rotation and crop management.

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