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Assessing the Pathogenic Ability of Six Species of *Fusarium* Genus on Onion Variety in Burkina Faso

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Abstract

Prema 178 onion variety is widely used in production in Burkina Faso. It is greatly appreciated but susceptible to basal rot. This study aimed to evaluate the pathogenic ability of six strains of *Fusarium* genus identified in Burkina Faso on onion. Seeds, seedlings and bulbs were used for the test. A conidial suspension of each strain was made in tubes and adjusted to 1×10^6 conidia/ml with distilled water for the different tests. Germination test in the laboratory and greenhouse showed that all treatments with the strains of *Fusarium oxysporum* f. sp. *cepae*, *F. solani*, *F. falciforme*, *F. acutatum*, *F. proliferatum* and *F. sp.* induced failure to emerge and showed a significant difference with the control. The different strains also induced stunting rates of coleoptile growth. *Fusarium oxysporum* f. sp. *cepae*, *F. acutatum*, *F. proliferatum*, *F. falciforme* and *F. solani* were very aggressive, as they recorded above 50% damping-off rates. The test on the bulbs revealed that the strains were classified into two groups. The first consists of *F. oxysporum* f. sp. *cepae*, *F. solani*, *F. falciforme*, *F. acutatum*, which caused rots with respective lengths from 2.06; 1.48; 1.84; 1.46 and 2.12 cm, thus very aggressive according to Ghanbarzadeh scale. The second is formed by *F. proliferatum* which recorded 0.90 cm of rot length, thus moderately aggressive. It would be appropriate to suggest a sustainable management method for these pathogens in order to improve the yield of onion production.

Keywords

Fusarium spp., Pathogenicity, *Allium cepa* L., Burkina Faso

1. Introduction

The onion (*Allium cepa* L.) is a commonly produced and used vegetable around the world. It is used as a basic component in many preparations. In West Africa it is also used as a raw ingredient in salads or as an addition to grilled meat [1]. World production of dried bulb onions was 96,773,819 in 2018 with an average yield of 19.20 t/ha [2]. In Burkina Faso, the onion production chain contributes significantly to the fight against food insecurity and poverty [3]. Onion production has indeed increased by about 50% in the space of ten years, from 242,258 tons in 2008 to 408,832 tons in 2017. However, it has experienced declines in recent years. It decreased from 408,832 tons in the 2016-2017 season on 16,850 ha to 362,480 tons in 2018-2019 on 18,491.49 ha [4]. The decrease of onions production is due to several factors, including poor soil fertility, inappropriate crop management practices and fungal diseases [3] [5] [6]. Among these diseases, basal rot caused by *Fusarium* fungi, can cause significant crop losses ranging from 2.9% to 80% [7]. Köycü [8] has reported that seeds and bulbs of onions and soil are the main source of inoculum of *Fusarium* fungi. Among the species of the *Fusarium* genus reported as agents of onion basal rot worldwide, *Fusarium oxysporum*, *Fusarium solani* and *Fusarium proliferatum* are the most common species encountered [9] [10]. In addition, it has been shown over the past decades that fungi frequently found in food can produce toxins, named mycotoxins. These toxins can cause human cancers. Mycotoxins such as fumonisins, trichothecenes and zearalenone are produced by some fungi of the *Fusarium* genus and are responsible for cancer [11]. In Burkina Faso, several fungal species of the *Fusarium* genus have been reported to cause *Fusarium* basal rot disease on onions [12] [13]. However, the pathogenicity of these fungi has not yet been documented. This study aims to investigate the pathogenic ability of six species identified on onion in Burkina Faso.

2. Materials and Methods

2.1. Onion Variety Used

Prema 178 variety was used for the experiment. It was selected for its sensitivity to Fusarium basal rot. Seeds, bulbs and seedlings were used for the different tests. Bulbs were obtained from a producer of onions who used this variety. This variety has a 170-day cycle, producing large red bulbs with a potential yield of 30 tons/ha. It has a rainy season crop vocation.

2.2. Fungi Isolation and Identification

Fungal material was composed of six species of Fusarium. These were *Fusarium solani*, *Fusarium oxysporum* f. sp. *cepae*, *Fusarium acutatum*, *Fusarium falciforme*, *Fusarium proliferatum* and *Fusarium sp.* These six strains were derived from an identification of *Fusarium* species collected on onion in Burkina Faso. Organ fragments of onion were surface sterilized briefly and washed using 3%

sodium hypochlorite and distilled water, respectively. The organ fragments were then used to obtain pure cultures of fungus isolates base on a repeating transfer technique using Potato Dextrose Agar (PDA) medium with an antibiotic commercially named spectinomycin. The obtained pure cultures of fungus isolates were used for the morphological and molecular identifications as describe below [13].

2.3. *Fusarium* Fungi Inoculum Preparation

For this experiment, we have used a seven-day-old culture of each *Fusarium* species, grown on potato dextrose agar (PDA) plate at 28˚C under a 12:12 light/dark photoperiod. After pouring five milliliters of sterile water on each plate, the mycelium and conidia were harvested by scraping the surface of the *Fusarium* colonies using a sterile scalpel. Then, the conidial concentration of the suspension obtained was determined using a Neubauer hemocytometer. The suspension was adjusted to the final concentration of 1×10^6 for the pathogenicity test on seeds, seedlings and bulbs [13].

2.4. *Fusarium* Species Effects on Seed Germination

The effects of *Fusarium* fungi on onion seed germination, seedlings and bulbs were assessed following the protocol described in [14]. After washing with distilled water, the onion seeds were immersed in 1% NaOCl for 10 min and then rinsed with distilled water for 15 min to ensure that fungi were not carried on the seed surface. A sample of 25 seeds of the onion variety Prema 178 was immersed in 10 ml of conidia suspension of a *Fusarium* species in a tube for 30 minutes. Then, the seeds were placed on sterile Whatman paper in a petri dish. The experiment was replicated five times. After nine days of incubation at 28˚C, the germination rate and the length of the coleoptiles were recorded (formula 1). The stunting rate was calculated according to the formula 2 [15] [16].

$$\text{germination rate} = \frac{\text{Number of germinated seeds}}{\text{Number of sowed seeds}} \times 100 \quad (1)$$

$$\text{Stunting rate} = \frac{\text{ACLC} - \text{ACLT}}{\text{ACLC}} \times 100 \quad (2)$$

ACLC = average coleoptile length of the control.

ACLT = average coleoptile length of the treatment.

2.5. Assessment of the Effects of *Fusarium* Strains on Seedlings in the Greenhouse

In this experiment, we have used pots containing a mixture of sterile sand and sterilized compost (bokashi) in a 1:3 ratio. Disinfected onion seeds in 1% NaOCl for 10 min were inoculated with the different *Fusarium* strains and then 20 seeds were sowed in each pot. Control seeds were immersed in distilled water. The experiment was replicated five times. Pots were placed in a greenhouse (25˚C -

30˚C, 50% RH) under 14:10 light/dark photoperiod, following the protocol of [17]. Watering was carried out with sterile water using a manuel sprayer. Fourteen days after sowing (DAS) we have assessed the emergence rate of seedlings, and the percentage of damping-off was calculated at 21 DAS and 45 DAS using the formula 2. Strains that caused damping-off rates above 50% were considered highly virulent [17].

2.6. Testing the Effects of *Fusarium* Strains on Onion Bulbs Rot

For this experiment, fifteen bulbs with a diameter between 3 to 3.5 cm and grown in an incubator set at 28˚C were used per treatment for this test. For each *Fusarium* species, conidia suspension was prepared. After removing the outer scales, the bulbs were sterilized in 70% ethanol solution for 30 seconds. Perforations approximately 5 mm deep were made in the basal plate with a 4 mm diameter sterile punch. A total of five small holes were made in each bulb. Ten microliters of each inoculum were introduced into the holes and sealed with tape. The same quantity of water was used for the controls. The experiment was replicated five times. Rot length was observed for each treatment after two weeks of incubation and was noted by measurement [18] [16].

The severity was classified into three groups:

- rot length between 0.2 - 0.6 cm, less aggressive.
- rot length between 0.6 - 1 cm, moderately aggressive.
- rot length over 1 cm, very aggressive.

2.7. Data Analysis and Results Expression

Germination rate and stunting were expressed in percent and in centimeters for coleoptile length. All data were submitted to analysis of variance based using the R software version 4.1.1. Comparisons of the different means with the control were made using a 5 % significance level Tukey test.

3. Results

3.1. Effects of *Fusarium* Strains on Onion Seeds Germination

Table 1 shows the effects *Fusarium* strains on the percentage of emergence A at 9 DAS and coleoptile length. Statistical analysis revealed that seeds inoculation with each *Fusarium* strain have significantly reduced the germination of seeds of onion compared to the control seeds ($p \leq 0.001$). The percentage of emergence was ranged from 33.60% to 56% for inoculated seeds and 84% for the control seeds. No differences were recorded between the effects of the *Fusarium* strains on onion seeds germination.

Regard to coleoptile growth, all *Fusarium* strains significantly decreased coleoptile length compared to the control ($p \leq 0.001$). According to the results of the statistical analysis, there was a significant difference between the effects of *Fusarium* strains on coleoptile growth ($p \leq 0.001$). The smallest coleoptile length

Table 1. Effect of *Fusarium* strains on seeds emergence and coleoptile emergence and length.

Fusarium species	Emergence 9 DAS (%)	Coleoptile length (cm)	stunted growth rates
Control	84.00 ± 5.66^{b}	3.71 ± 0.45^{c}	-
Foc	39.20 ± 8.67^{a}	0.71 ± 0.17^{a}	79.87 ± 8.50^{c}
Fs	33.60 ± 6.07^{a}	1.21 ± 0.44^{ab}	67.38 ± 7.00^{ac}
Ff	44.00 ± 11.31^{a}	0.92 ± 0.42^{ab}	73.84 ± 3.00^{c}
Fa	52.80 ± 15.07^{a}	1.88 ± 0.47^{b}	49.30 ± 9.00^{a}
Fp	56.00 ± 13.56^{a}	1.79 ± 0.50^{b}	51.77 ± 1.00^{ab}
Foc	37.60 ± 18.24^{a}	1.14 ± 0.67^{ab}	69.10 ± 9.00^{bc}
p-value	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$

Note: Averages not sharing any letters are significantly different at the 5% level, according to the Tukey test. DAS = days after sowing; Foc = *F. oxysporum* f. sp. *cepae*; Fs = *F. solani*; Ff = *F. falciforme*; Fa = *F. acutatum*; Fp = *F. proliferatum*; Fsp = *F. sp.*

(0.71 cm) was recorded with seeds inoculated with *F. oxysporum* f. sp. *cepae* compared to seeds inoculated with *F. acutatum* (1.88 cm) or *F. proliferatum* (1.79 cm).

All strains induced stunted coleoptile growth rates. Rates ranged from 49.30% to 79.87%. A significant difference was noted ($p \leq 0.001$). *F. oxysporum* f. sp. *cepae* and *F. falciforme* treatments recorded the highest reduction rates compared to *F. acutatum*.

3.2. Effects of *Fusarium* Strains on Onion Seedlings and Root Length in Greenhouse

Emergence at 14 DAS and root length at 45 DAS are presented in Table 2. Emergence rate ranged from 32% to 53% for inoculated seeds and 73% for the control seeds. There was a highly significant difference between the emergence rate in inoculated seeds and the emergence rate in the control seeds ($p \leq 0.007$). However, no significant difference was recorded between the *Fusarium* strains used.

The root length ranged from 3.04 to 3.50 cm for the strains of *Fusarium* compared to 4.50 cm with the control. No significant difference was recorded between the treatments ($p \leq 0.631$).

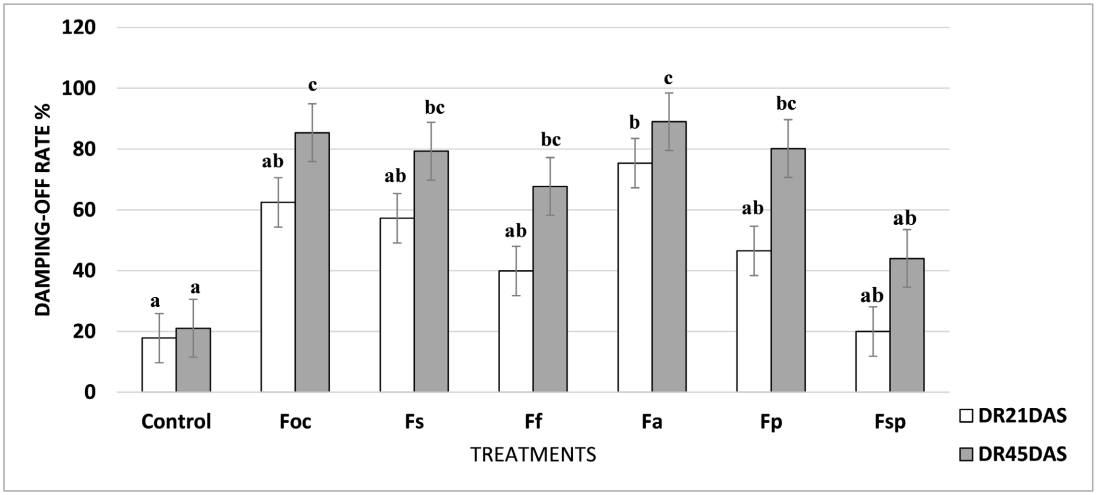
3.3. Effects of *Fusarium* Strains on Onion Seedlings Damping-Off in Greenhouse

Figure 1 shows the damping-off rates at 21 and 45 DAS. At 21 DAS damping-off was observed and some plants showed similar symptoms to those observed in the field during the survey. No symptoms were observed on the control plants. *Fusarium* strains used for inoculation were re-isolated from root trays of diseased

Table 2. Emergence rate and root length of inoculated seedlings.

Fusarium species	Emergence at 14 DAS (%)	Roots length (cm)
Control	73 ± 10.37[b]	4.50 ± 0.65
Foc	53 ± 16.81[ab]	3.04 ± 1.11
Fs	34 ± 4.18[a]	3.40 ± 2.00
Ff	32 ± 4.47[a]	3.20 ± 1.58
Fa	40 ± 10.61[a]	3.10 ± 1.41
Fp	45 ± 20.31[a]	3.50 ± 1.14
Foc	35 ± 14.14[a]	3.20 ± 0.84
p-value	≤0.007	=0.631

Note: Averages not sharing any letters are significantly different at the 5% level, according to the Tukey test. DAS = days after sowing; Foc = *F. oxysporum* f. sp. *cepae*; Fs = *F. solani*; Ff = *F. falciforme*; Fa = *F. acutatum*; Fp = *F. proliferatum*; Fsp = *F. sp.*



Note: Sticks not sharing any letters are significantly different at the 5% level, according to the Tukey test. DR = Damping-off; Foc = *F. oxysporum* f. sp. *cepae*; Fs = *F. solani*; Ff = *F. falciforme*; Fa = *F. acutatum*; Fp = *F. proliferatum*; Fsp = *F. sp.*

Figure 1. Damping-off rate at 21 DAS and 45 DAS of Prema 178 variety according to the treatments.

plants to check Koch's postulate. Damping-off was observed for all treatments at this time. Nevertheless, the highest damping-off was recorded in seedlings from inoculated seeds. Statistical analysis has revealed significant difference between the damping-off rate in seedlings from inoculated seeds and that in control seedlings at 21 DAS ($p \leq 0.002$) and 45 DAS ($p \leq 0.001$). *F. acutatum* showed a significant difference with the control at 21 DAS ($p \leq 0.006$). *Fusarium oxysporum* f. sp. *cepae*, *F. solani*, *F. falciforme*, *F. acutatum* and *F. proliferatum* recorded significant differences with the control with $p \leq 0.001$, $p \leq 0.001$, $p \leq 0.006$, $p \leq 0.001$ and $p \leq 0.001$ respectively. *Fusarium sp.* did not show any significant difference with the control. According to the scale of Bayraktar and Do-

lar [17], *Fusarium oxysporum* f. sp. *cepae*, *F. acutatum*, *F. proliferatum*, *F. falciforme* and *F. solani* strains were very aggressive. In contrast, Fusarium sp. was not aggressive.

3.4. Effects of *Fusarium* Strains on the Rot of Onion Bulbs

All strains of *Fusarium* have caused the rot in onion bulbs (**Table 3**). Statistical analysis indicated that there was a significant difference between the rot lengths caused by *Fusarium* species ($p \leq 0.001$). *F. oxysporum* f. sp. *cepae*, *F. falciforme* and *Fusarium sp.* have caused the deepest onion bulb rots, 2.06 cm, 1.84 cm and 2.12 cm, respectively. The rot lengths allowed to group *Fusarium* strains into two groups according to the scale of [17]. Thus, *F. oxysporum* f. sp. *cepae*, *F. solani*, *F. falciforme*, *F. acutatum* and *Fusarium sp.* which caused rot lengths from 1.46 to 2.12 cm were classified as very aggressive, and *F. proliferatum* with a rot length of 0.90 cm, as moderately aggressive. **Figure 2** shows evidence of onion bulb rot due to *Fusarium* strains. No rot was observed on the control bulbs (**Figure 2(a)**). *Fusrium oxysporum* f. sp. *cepae*, *Fusarium falciforme* and Fusarium sp. induced rotting of scaly leaves and the fleshy leaves, while the bud remained healthy (**Figures 2(b)-(d)**). *Fusarium acutatum* and *Fusarium solani* induced slight rotting of scaly leaves and the fleshy leaves (**Figure 2(e)**, **Figure 2(f)**). *F. proliferatum* showed the start of rot of scaly leaves and the fleshy leaves (**Figure 2(g)**).

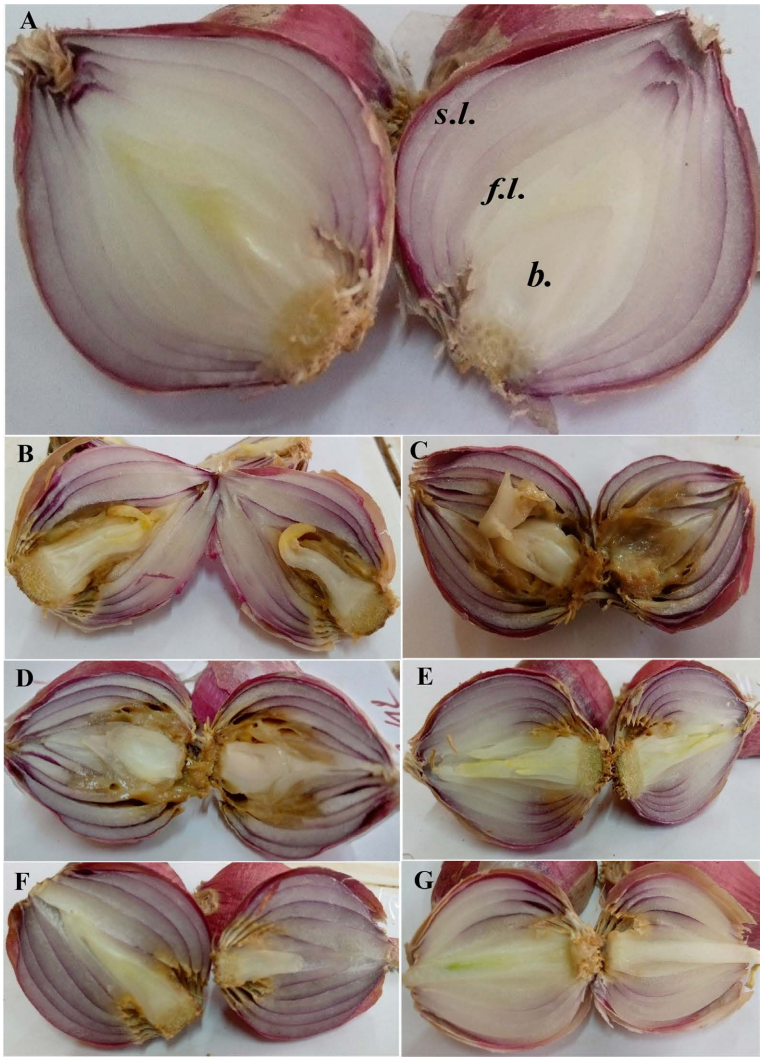
4. Discussion

Pathogenicity test performed on the onion Prema 178 variety allowed to discriminate the strains on a number of parameters and to compare their degree of pathogenicity with the control. The results obtained showed that all the *Fusarium* fungal strains had an inhibitory effect on onion seeds emergence. This

Table 3. Rot length (in cm) caused by the fungal strains.

Fusarium species	Rots length (cm)
Control	0.14 ± 0.05^{a}
Foc	2.06 ± 2.06^{d}
Fs	1.48 ± 0.11^{c}
Ff	1.84 ± 0.21^{d}
Fa	1.46 ± 0.22^{c}
Fp	0.90 ± 0.14^{b}
Foc	2.12 ± 0.08^{d}
p-value	$P \leq 0.001$

Note: Averages not sharing any letters are significantly different at the 5% level, according to the Tukey test. Foc = *F. oxysporum* f. sp. *cepae*; Fs = *F. solani*; Ff = *F. falciforme*; Fa = *F. acutatum*; Fp = *F. proliferatum*; Fsp = *F. sp.*



Note: A = Control; B = *F. oxysporum* f. sp. *cepae*; C = *F. falciforme*; D = *Fusarium sp.*; E = *F. solani*; F = *F. acutatum*; Fp = *F. proliferatum*; s.l. = scaly leaves; f.l. = fleshy leaves; b. = bud.

Figure 2. Rots caused by the strains on Prema 178 bulbs variety.

effect was observed in the laboratory and in the greenhouse. This would confirm the pathogenicity of the strains used in the present study. Indeed, several species of the genus *Fusarium* are known to be pathogenic fungi of onion. Kalman [16] and Tirado-Ramirez [19] reported that *F. acutatum* and *F. falciforme* can prevent onion seed germination. Similarly, [20] [21] and [22] also showed that *F. oxysporum* and *F. proliferatum* could cause failure to emerge and damping-off. *Fusarium* can be transmitted to onions during all stages of development and will develop under remarkably broad conditions [23] [24].

All strains used showed an inhibitory effect on coleoptile growth, thus causing the observed growth retardation rates. This growth inhibition attests to the pathogenicity of the species tested and their impact on seedling emergence. Indeed, during plant growth, coleoptile helps first leaf emergence (and shoot apex) by

protecting it during it passage in the soil. The first leaf breaks through the tip of the coleoptile at emergence. In addition to stunting, damping-off was observed in all the fungal strains tested. Several authors have reported the action of species of *Fusarium* genus as damping-off fungi individually or in combination. This action synergy was reported by some authors [25] [22] [26] [27]. *Fusarium* pathogens use both general and specific pathogenicity to invade their hosts. Hydrolytic enzymes involved in plant cell wall damage and cell signaling pathway components, which are often required for systemic pathogen invasion, are comprising pathogenicity factors, while the production and secretion of effectors and host-specific toxins are specific pathogenicity factors [28]. *Fusarium sp.* strain did not differ from the control in damping-off. However, in the laboratory and in the greenhouse, it did cause a lack of emergence. This would indicate that the strain of this species used would not be a pathogen associated with damping-off. No difference was found in root length between the strain and control treatments when measured at 45 days. That could be explained by the fact that the plants that were able to survive until 45 days corresponding to the end of the nursery period developed resistance to the pathogen strains used and started to grow well.

Based on the results of the bulb infection, it was possible to group the strains into two groups according to the aggressiveness, which was reflected in the length of the rots observed. The first group is constituted by *F. oxysporum* f. sp. *cepae*, *F. solani*, *F. falciforme*, *F. acutatum*, and *Fusarium sp*. which were the most aggressive. The second group was *F. proliferatum* which was considered moderately aggressive. This work is in agreement with [19]. They report in their work that *F. falciforme* is a new agent of onion bulb rot and would be more aggressive on the bulb than *F. oxysporum*. Kintéga [22] showed that there are strains of *F. proliferatum* which are very aggressive on bulbs and others which are less aggressive, which could justify the results obtained in this study which show that *F. proliferatum* was not very aggressive. Ghanbarzadeh [18] reported that *F. solani* is a rotting agent of underground parts of onion.

In this study, the aggressiveness was variable according to the strain and the organ targeted for inoculation. Taylor [29] found correlations between the pathogenicity on onion seedlings and bulbs of some *Fusarium* species. This work reported the pathogenicity of *Fusarium* strains isolated in Burkina Faso, which could affect onion production in this country. Data on the pathogens responsible for onion basal rot disease are valuable, as they can contribute to the development of breeding programs for resistant cultivars and control methods.

5. Conclusion

The pathogenicity study on six strains of the *Fusarium* genus (*F. oxysporum* f. sp. *cepae*, *F. solani*, *F. falciforme*, *F. proliferatum*, *F. acutatum* and *Fusarium sp.*) revealed that all strains of these species are pathogenic and induce emergence failures and have an inhibitory effect on coleoptile growth. In contrast to the

Fusarium sp. strain, all other strains cause severe damping-off. The bulb rot test allowed the strains of these species to be classified into two groups. The first group is constituted by *F. oxysporum* f. sp. *cepae*, *F. solani*, *F. falciforme*, *F. acutatum*, and *Fusarium sp*. and the second group by *F. proliferatum*. In this study, a variable pathogenicity of the tested strains was observed, the species behaved differently depending on the inoculated organ. It would be appropriate to suggest a sustainable management method for these pathogens in order to improve the yield of onion production.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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