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Sustainable Control of *Fusarium verticillioides* in Wheat Using Plant Extracts and Microorganisms

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Abstract

Fusarium verticillioides is frequently reported as the major pathogen in maize production; however, it has not been previously identified as a causal agent of Fusarium head blight in wheat in Algeria. The aims of this work are to study the pathogenicity of a *Fusarium verticillioides* strain isolated from wheat grains and to perform preliminary assays to control this pathogen using plant extracts from *Rosmarinus officinalis*, *Origanum vulgare* and *Eucalyptus globulus*, and three microorganisms *(Akanthomyces muscarius*, *Pseudomonas fluorescens*, and *Pantoea agglomerans)*. The identity of the strain was confirmed using morphological and molecular methods. The pathogenicity tests were carried out using both durum and bread wheat grains. The agar dilution method was used to evaluate the antifungal activity of the extracts, while the direct confrontation method was used to assess the antifungal activity of the three microorganisms. The results showed that the strain was pathogenic to both durum and bread wheat. Aqueous extracts showed low inhibition rates (14 to 47%), while ethanolic extracts had higher inhibition rates (52 to 65%). Direct confrontation tests revealed inhibition rates of 55% and 52% for *A. muscarius* and *P. fluorescens*, respectively. The fungus *A. muscarius* was also shown to be able to reduce the symptoms of *F. verticillioides* in antagonism tests using wheat seeds. The ethanolic extracts of the three plants, *A. muscarius*, and *P. fluorescens* may offer alternative solutions to the use of fungicides.

Keywords
Fusarium verticillioides, plant extracts, *Akanthomyces muscarius*, *Pseudomonas fluorescens*

1. Introduction

Wheat is susceptible to attacks by various phytopathogenic fungi in all stages of growth, from seedling to maturity (Pastuszak et al., 2021). Species of the genus *Fusarium* are among these fungi. They are responsible for two important diseases in wheat; the first is Fusarium crown rot (FCR), which causes root system and crown rotting and precocious maturing with sterile white heads. It is mainly caused by *Fusarium acuminatum*, *F. avenaceum*, *F. equiseti*, *F. crookwellense* and *F. culmorum* (Campanella, 2023). The second disease is Fusarium head blight (FHB) causing an important crop loss and reduction of harvested grains quality. The most aggressive FHB species are *F. culmorum*, *F. graminearum* and *F. avenaceum*; while other species are less frequent and less aggressive, such as *F. cerealis*, *F. poae*, *F. equiseti*, *F. solani*, *F. verticillioides* and *F. proliferatum* (Sakr, 2022).

F. vertillicioides is frequently reported as the major pathogen in maize production (He et al., 2023). It has been associated with several diseases in maize for almost a century, including root rot, stem rot and seedling blight (Yates et al., 2005). In wheat production; however, it was rarely reported as a pathogen or as a grain contaminant. But in the last ten years, it was reported as an emergent pathogen in Finland (Gagkaeva and Yli-Mattila, 2020), as species associated with FHB in South Russia (Gagkaeva et al., 2018) and species associated with FCR in Algeria (Abdallah-Nekache et al., 2019).

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The use of synthetic fungicides remains the main tool to control *Fusarium* diseases on wheat (Bachouche et al., 2024). Fungicides used are mainly triazoles like hexaconazole, propiconazole, tebuconazole and triadimenol. Other chemicals can also be applied, such as carbendazim and prochloraz (Dweba et al., 2017; Zhao et al., 2021). However, none of these chemicals provides a complete protection against wheat *Fusarium* diseases (Dweba et al., 2017).

The development of alternative methods to the use of synthetic fungicides has become a necessity in order to better protect crops, environment and humans' health (Seepe et al., 2020). The biocontrol of *Fusarium* by the use of microorganisms such as bacteria and fungi has already been reported by several authors. Dweba et al. (2017) and Matarese et al. (2012) recommended *Brevibacillus* sp. (strain BRC 263), *Streptomyces* sp. (strain BRC87B) and *Trichoderma gamsii* (strain 6085) as potential biocontrol agents of *Fusarium* disease in cereal. *Bacillus* sp. (strain B25) are also effective biocontrol agents against maize *Fusarium* disease (Douriet-Gámez et al., 2018). The biocontrol of *Fusarium* by using the bioactive compounds extracted from plants has been also studied. Dambolena et al. (2012) reported that natural phenolic compounds such as carvacrol, thymol and isoeugenol have a high antifungal activity against *F. verticillioides.* Seepe et al. (2020) studied aqueous and organic extracts of thirteen medicinal plants from South Africa. They found that acetone extracts obtained from *Quercus acutissima*, *Harpephyllum caffrum* and *Combretum erythrophyllum* are the most effective extracts inhibiting the growth of *F. proliferatum, F. graminearum, F. solani* and *F. verticillioides* with minimum inhibitory concentration values less than 0.1 mg/ml. They found also that aqueous extracts of *Olea europaea, Withania somnifera, Combretum molle* and *Lantana camara* are effective and can be used to manage diseases caused by *Fusarium* species.

Based on the aforementioned reports, we hypothesize that plant extracts and beneficial microorganisms can provide an effective approach to controlling *F. verticillioides* while minimizing environmental impacts. The use of plant extracts and microorganisms to combat *F. verticillioides* can significantly reduce reliance on synthetic fungicides, thereby decreasing their undesirable negative effects. These effects might include impacts on non-target organisms and thus on biodiversity, and persistence in the environment. Indeed, while beneficial microorganisms and plant extracts are not entirely specific, their spectrum of action remains much narrower compared to synthetic fungicides (Fenta et al., 2023). Moreover, certain microorganisms, such as *Pseudomonas fluorescens*, can contribute to increasing soil fertility (Martínez et al., 2019). Additionally, plant extracts have the advantage of being biodegradable in the soil. However, the use of exogenous antagonistic microorganisms not native to the treated area can cause significant disruptions to the ecosystem and local microbial populations. The implementation of control methods based on plant extracts and antagonistic microorganisms should be preceded by thorough risk assessments and field trials to guarantee their effectiveness, safety, and optimal application.

The aims of this work are to study the pathogenicity of a *Fusarium verticillioides* strain isolated from wheat grains and to perform preliminary assays to control this pathogen using aqueous and ethanolic extracts of three plants *(Rosmarinus officinalis, Origanum vulgare, Eucalyptus globulus),* the fungus *Akanthomyces muscarius*, and two bacteria *(Pseudomonas fluorescens* and *Pantoea agglomerans).*

2. Data and methods

2.1. Isolation and morphological identification of *Fusarium verticillioides*

The durum wheat seeds (20 samples) used for isolation in this study were provided by the Cooperative of Cereals & Pulses (CCLS) of Bouira Province, Algeria. All the samples came from Bouïra fields characterized by a semi-arid climate. The isolation was performed using the protocol of Ioos et al. (2004). Briefly, wheat grains were superficially disinfected by soaking them in a solution of sodium hypochlorite at 1.5 V/V Cl with the addition of Tween 20 (0.01 V/V%) for 10 minutes. The grains were then placed to dry on sterile filter paper. Among the perfectly dried grains, at least 100 grains were taken at random and placed directly on Dichloran Chloramphenicol Peptone Agar (DCPA) medium described by Andrews and Pitt (1986) at the rate of 5 grains per Petri plates. DCPA Petri plates were incubated for 10-12 days at 25 °C.

Typical *Fusarium* colonies were subsequently transferred to PDA (Potato Dextrose Agar) and SNA (Synthetischer Nahrstoffarmer Agar – Synthetic Nutrient Deficient Agar) and were identified after 5 to 10 days of incubation at 25 °C following the protocol of Leslie and Summerell (2008). *F. verticillioides* was detected in two samples.

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2.2. Molecular identification and phylogenetic analysis

Genomic DNA was extracted from monosporic *F. verticillioides* S03 strain culture, using a commercial NucleoSpin Plant II kit (Macherey-Nagel, Germany) according to the manufacturer's recommendation. For the S03 strain, segments of ITS (Nuclear ribosomal Internal Transcribed Spacer) region were amplified by the primer pairs ITS1 (CTT GGT CAT TTA GAG GAA GTA A) and ITS4 (TCC TCC GCT TAT TGA TAT GC) as described by Gardes and Bruns (1993); also, segments of TEF-1α (Translocation Elongation factor 1-alpha) region were amplified using the primer pairs EF-728F (CAT YGA GAA GTT CGA GAA GG) and EF2 (GGA RGT ACC AGT SAT CAT GTT) according to Carbone and Kohn (1999). Amplification was performed with a total volume of 25 µL, containing 2 µL of genomic DNA, 1µL of 10µM primers, 5µL of 5X Taq buffer (Promega Corporation, USA), 1.5 µL of 25 mM $MgCl_2$, 0.2µL of d'NTP mixture (25 mM), 0.2µL of 5U/µL of Taq DNA polymerase (Promega Corporation, USA), and 14.10 µL of double-distilled sterile water. PCR conditions for the ITS region were as follow: Initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 30 s, hybridization temperature of 55 °C for 30 s, extension at 72 °C for 45 s, followed by a final extension at 72 °C for 7 min. The same conditions were used for the TEF1-α region; however, the hybridization temperature was changed to 52 °C. After the PCR reaction, the PCR product was separated into a 1.5% agarose gel (Sigma-Aldrich, USA) and stained with 0.5µg ml^{-1} ethidium bromide. A molecular weight marker of 100 bp (PCR 100 bp low Ladder, Sigma-Aldrich) was used. DNA fingerprints were visualized following exposure to UV light using the Gel Documentation System (Bio-Rad, USA). The PCR products were then purified using the NucleoSpin® Gel and PCR Clean-up kit from Macherey-Nagel (Germany) and sequenced by the Sanger technique using the BigDye v3.1 kit from Applied Biosystems and the same primers used in PCR. The obtained sequences were analyzed and corrected using the CHROMAS PRO software.

The obtained sequences have been compared with those in the National Center for Biotechnology Information (NCBI) database using the programme BLAST. The S03 strain was identified based on percentage homology with published ITS and TEF-1α sequences. Phylogenetic tree has been generated by TEF-1α sequences alignment using MEGA7 software. Maximum likelihood analyzes (ML) were applied using 54 TEF-1α sequences from *Fusarium fujikuroi* species complex. Two sequences from *F. oxysporum* NRRL 22902 and *F. inflexum* NRRL20433 were used as out group. The support of the internal branches of the tree was evaluated by the bootstrap method with 1000 replications.

2.3. Pathogenicity test

In order to evaluate the pathogenicity of *Fusarium verticillioides* strain, seeds of two species of wheat (Bread wheat: cultivar of HD1220; Durum wheat: cultivar of Oued El Bared) were used. For each wheat species and for each treatment, a number of 100 seeds were surface-sterilized by soaking in a 2% NaClO solution for 8 min, then rinsed twice with sterile distilled water. The seeds were placed in Petri plates lined with sterile absorbent paper in triple layer at the rate of 25 seeds per Petri plate. For inoculum preparation, the protocol of Bouanaka et al. (2022) was adopted. Each *Fusarium* strain was cultured on PDA medium at 26 °C. for 15 days. A volume of 10 ml of sterile distilled water with 0.01% (v/v) of Tween-80 was added to the aerial part of *F. verticillioides* 15day-old culture. The culture surface was then carefully scraped until the entire superficial layer of the fungus had been recovered. The resulting suspension, consisting of mycelium and conidia, was filtered through a double layer of sterile cheesecloth and adjusted to 10^6 conidia ml^{-1} using sterile distilled water with 0.01% (v/v) of Tween-80. A volume of 5 ml of the inoculum suspension was sprayed onto each Petri plate containing wheat seeds. Petri dishes were then incubated at 26 °C in total darkness (Bouanaka et al., 2022). Control plates were prepared in the same way by substituting the inoculum suspension with sterile distilled water with 0.01% (v/v) Tween-80. The rate of the seeds germination, the length and the weight of coleoptile were calculated four days after the inoculation.

2.4. Antifungal activity of plant extracts against *F. verticillioides*

Rosemary *(Rosmarinus officinalis)*, oregano *(Origanum vulgare)* and eucalyptus *(Eucalyptus globulus)* tested were from Bouira Province. Fresh leaves were collected in the April–May period and shade-dried at room temperature. The dried leaves were ground into fine powders using a coffee grinder. Aqueous extracts were obtained by soaking 15 g of leaf powder in 150 ml of distilled water for 24 h with stirring. The extracts were filtered through Whatman No. 1 filter paper and stored at 4 °C. Ethanolic extracts were prepared using a Soxhlet apparatus with 15 g of leaf powder and 150 ml of ethanol (96°V/V%) were used. The extraction was carried out at 70 °C under atmospheric pressure for 3h.

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The agar dilution method was used to evaluate the antifungal activity of the extracts (Wilkinson, 2006). For aqueous extracts, a volume of 20 ml were incorporated into PDA medium to prepare 100 ml of final medium volume. For ethanolic extracts, an aliquot of 20 ml of each extract were evaporated using a rotary evaporator and then resolved in 20 ml of water and incorporated into PDA medium to prepare 100 ml of final medium. These media were sterilized at 120 °C for 20 min, and then were poured in sterile Petri dishes.

In order to compare antifungal activity of extracts, two synthetic fungicides were incorporated into the PDA medium in the same way as for the plant extracts. The first was Iprodione at concentration of 7.5 mg per 100 ml of PDA medium: it had been prepared from commercial wettable powder product containing 50% of Iprodione (50% WP). The second was Difenoconazole at 1.5 mg/ 100 ml of PDA medium; it had been prepared from commercial emulsifiable concentrate product containing 25% of Difenoconazole (25% EC)

For inoculation, agar discs (5 mm in diameter) were taken from ten days old culture plates of *F. verticillioides* and then placed on PDA Petri prepared with leaves extracts or with fungicides. The plates were incubated at 26 °C ± 2 °C. The control was carried out under the same conditions without leaves extracts or fungicides. The radial growth was then measured and compared with the control (Wilkinson, 2006). The inhibition percentage of growth was calculated according to this formula (1):

$$\text{Inhibition percentage} = (D_{control} - D_{test}) / D_{control} \times 100 \quad (1)$$

Where $D_{control}$ is the diameter of *F. verticillioides* colony in the control plates, D_{test} is the diameter of *F. verticillioides* colony in the plates amended with plant extracts or fungicides.

2.5. Antifungal activity of *Akanthomyces muscarius, Pseudomonas fluorescens, Pantoea agglomerans* against *F. verticillioides*

The strains of *Akanthomyces muscarius* (TA01), *Pseudomonas fluorescens* (Ps27), *Pantoea agglomerans* (B21*)* used in this study were offered by the Plant Microbiology Laboratory of Bouira University.

The antifungal assay of *A. muscarius* against *F. verticillioides* was carried out using direct confrontation method (Hibar et al., 2005). Agar discs (5 mm in diameter) were taken from ten days old culture plates of both fungi and then placed on new PDA Petri; these discs were placed 3 cm apart from each other. As a control, *F. verticillioides* agar disc alone was placed in the center of PDA Petri plate. The fungi were then incubated at 25 °C. The antifungal assays of the two bacteria were carried out using direct confrontation method (Girish and Bhavya, 2018), mycelial disc (5 mm in diameter) of ten days old culture of *F. verticillioides* was placed in the center of PDA plates, bacterial colonies of 48 h old culture were streaked 2.0 cm apart from the fungal discs on both the sides separately. PDA plates inoculated only with *F. verticillioides* served as control.

Radial growth of *F. verticillioides* was measured daily. The inhibition percentage of growth was calculated according to this formula (da Silva et al., 2017)(2):

$$\text{Inhibition percentage} = (\text{D control} - \text{D test}) / \text{D control} \times 100 \quad (2)$$

Where D control is the diameter of *F. verticillioides* colony in the control plates, D test is the diameter of *F. verticillioides* colony in the direction of the antagonist colony in dual culture plates.

2.6. Antagonism tests of *A. muscarius* using wheat seeds

This test was carried out using seeds of two species of wheat (Bread wheat: cultivar of HD1220; Durum wheat: cultivar of Oued El Bared). The disinfection of the wheat seeds and the preparation of the inoculum were carried out in the same way as in the pathogenicity test. Except that for the *F. verticillioides* inoculum the final concentration was adjusted to 2×10^6 conidia ml^{-1}. For *A. muscarius*, the suspension of conidia was prepared in the same way as for *F. verticillioides*. The final concentration was adjusted to 2×10^6 conidia ml^{-1}.

In each Petri plate containing 25 disinfected wheat seeds, 2.5 ml of antagonist suspension and 2.5 ml of *F. verticillioides* suspension were sprayed at the same time. Control plates were prepared in the same way by substituting *F. verticillioides* inoculum suspension with sterile distilled water with 0.01% (v/v) Tween-80. The notations (germination percentage, length and weight of coleoptiles) are taken after incubation at 26 °C for four days.

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2.7. Statistical analysis

Analysis of variance (ANOVA), comparison of means using LSD at the 5% threshold were performed using XLSTAT 2017 software. The graphs were generated using GraphPad Prism 8 software.

3. Results and discussion

3.1.Morphological and molecular identification of *F. verticillioides*

On a PDA medium, colonies of S03 strain grew rapidly (reach 8.3 ± 0.4 cm in 10 days). Mycelium in young cultures was white and turned purplish pink in old cultures. The medium under the culture was pink and became dark purple with age. On SNA medium, microconidia were unicellular oval to club-shaped with a flattened base. They were always formed from monophialides, and were found in long chains. Microconidia size was 6–14 × 2–3.5 µm (Figure 1). Macroconidia were not found in our study. Chlamydospores were absent.

The ITS sequence of S03 strain (Accession: OR610687) showed 99-100% similarity with several strains of *F. verticillioides* available in the NCBI database. However, it also showed high similarity percentages with other species among *Fusarium fujikuroi* species complex such as *F. subglutinans* and *F. fujikuroi.*

The highest percentage of genetic similarity of S03 based on the TEF-1α sequence (Accession: OR611969) was observed within *F. verticillioides*. Of the 100 closest sequences, 98 sequences were within *F. verticillioides.* TEF-1α sequences alone were effective in discriminating *F. verticillioides* from others species with morphological similarity within *Fusarium fujikuroi* species complex such as *F. fujikuroi*., *F. proliferatum, F. sacchari* and *F. nygamai* (Figure 2).

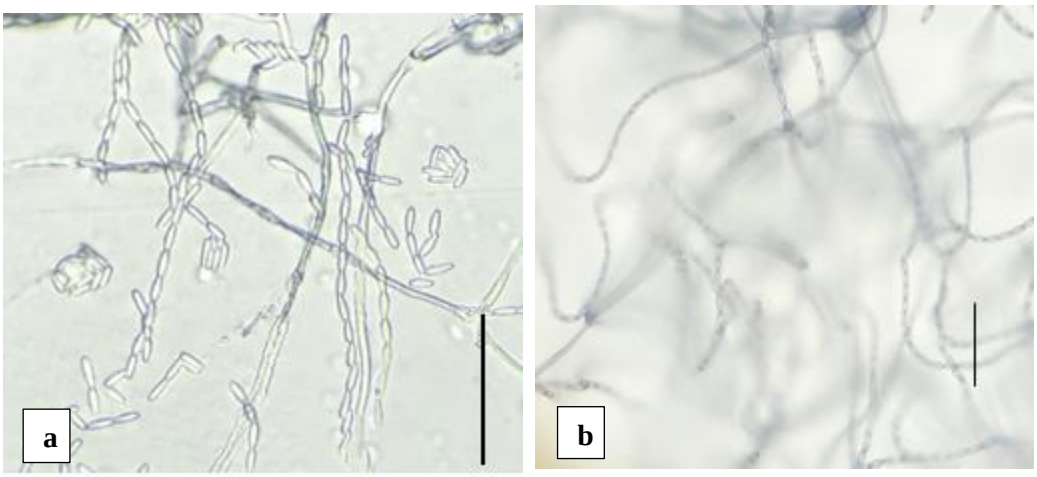

Figure 1. Microconidia *of F.verticillioides* in the slide mount (a) and in *situ* on SNA medium (b), scale bar = 50 µm

Fusarium head blight and Fusarium crown rot are two important diseases caused by the genus *Fusarium* in wheat. In Algeria, the species primarily responsible for Fusarium head blight are *F. culmorum* and, to a lesser extent, *F. pseudograminearum*. The species associated with Fusarium crown rot include *F. culmorum*, *F. avenaceum*, *F. pseudograminearum*, *F. chlamydosporum*, *F. equiseti*, *F. verticillioides*, and *F. cerealis*, with *F. culmorum* being the dominant species (Abdallah-Nekache et al., 2019; Bouanaka et al., 2022). Notably, *F. verticillioides* has not been previously cited as a causal agent of Fusarium head blight in wheat in Algeria.

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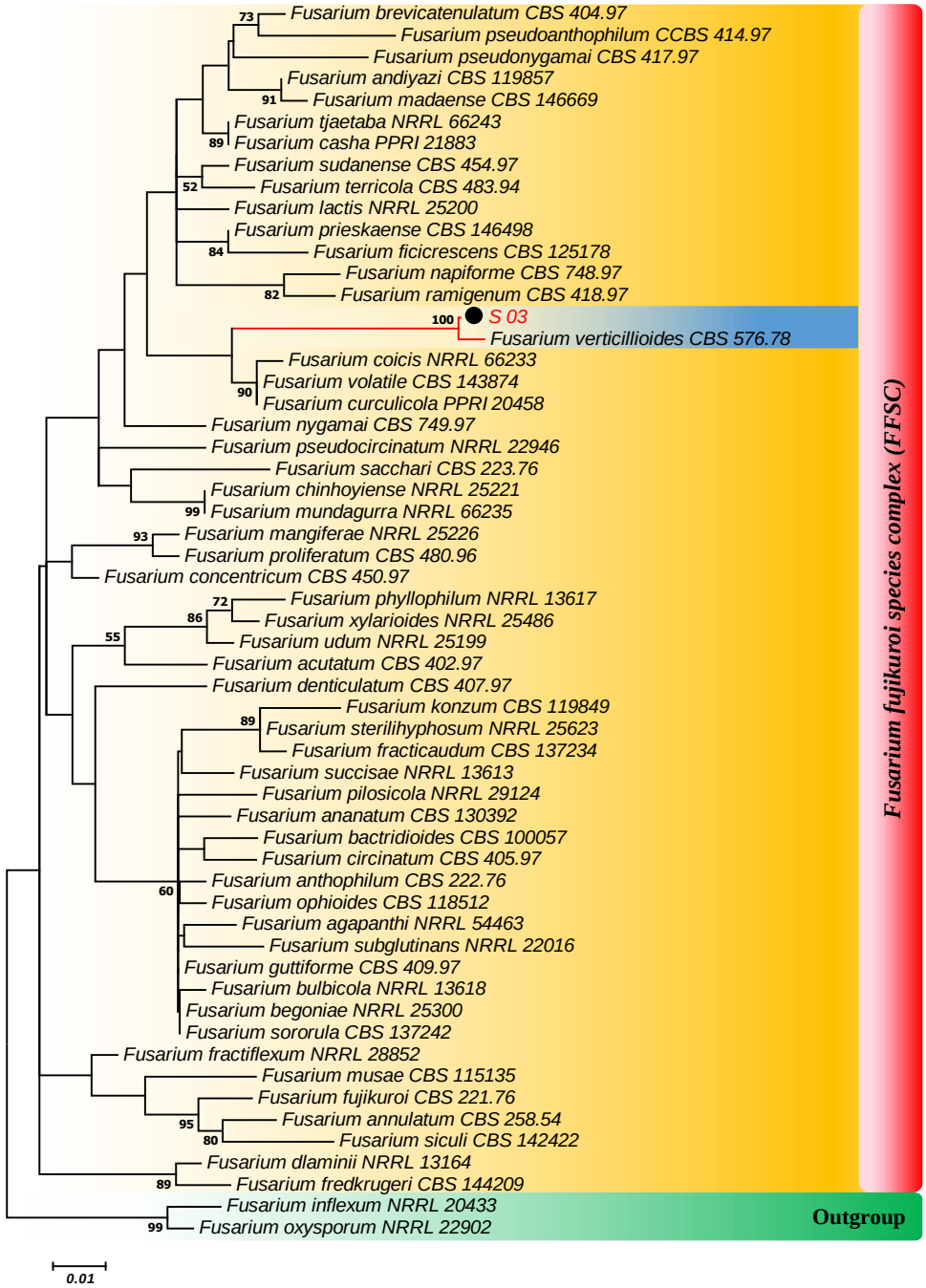

Figure 2. Phylogenetic tree generated from TEF-1α sequences: Numbers on the branches are percentages of 1000 bootstrap replications of MEGA6-maximum likelihood. Maximum likelihood bootstrap values MLBS ≥ 50 % are given at the nodes

In this study, we isolated a strain of *F. verticillioides* from two samples of wheat grain. The morphological characteristics of our strain closely resemble those described by Gagkaeva and Yli-Mattila (2020) who studied *F. verticillioides* newly emerged from winter wheat. Their isolates formed colonies with an average diameter of 58.1–65.8 mm after seven of days incubation on PSA medium at 25 °C. Aerial mycelium was white to violet. Microconidia were formed in chains from simple phialides, with a size range of 5–10 × 1.5–2.8 µm. Fusiform macroconidia were also observed. Sporodochia and chlamydospores were absent. In their study, molecular identification was also performed using TEF-1α sequences, and the isolates were identical with several known *F. verticillioides* isolates in GenBank.

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3.2. Results of pathogenicity test

The results of pathogenicity tests are presented in Table 1. The germination rates of durum and bread wheat seeds were 96% and 97%, respectively. However, when they were inoculated by *F verticillioides* spores suspension, these rates became 4.0% and 3.1% respectively (Table 1). Spore inoculation also caused a strong reduction on coleoptile length and weight in both durum and bread wheat.

Our strain was found to be pathogenic to both durum and bread wheat, causing a severe reduction in grain germination rates as well as coleoptile weight and length. These findings are consistent with the work of Sakr (2018) who reported a reduction in germination rate and coleoptile length in wheat grains inoculated with four isolates of *F. verticillioides* in Syria.

3.3. Effect of plants extracts and fungicides on radial growth of *F verticillioides*

The diameter of *F. verticillioides* colonies grown on PDA medium alone (control) and PDA medium supplemented with aqueous extracts, ethanolic extracts, and fungicides on the 4th and 7th days of incubation is presented in Figure 3. In the presence of aqueous extracts, a reduction of colony diameter was observed both in 4th and 7th day of incubation, which corresponds to an inhibition rate of 47 ± 2.5% in the case of aqueous oregano extract, 24.5 ± 3.5% in the case of aqueous eucalyptus extract, and 14 ± 4.6% in the case of aqueous rosemary extract. In the presence of ethanolic extracts, a more important reduction of mycelium growth was observed with an inhibition rate of 63.4 ± 2.4%, 57.7 ± 2.6 and 65.2 ± 2.4% in the case of extracts of oregano, eucalyptus and rosemary, respectively (Figure 3). On the other hand, the inhibition rates of mycelium growth recorded with fungicides were 49.8 ± 2.7% and 77.5 ± 2.5% for iprodione and difenoconazole, respectively.

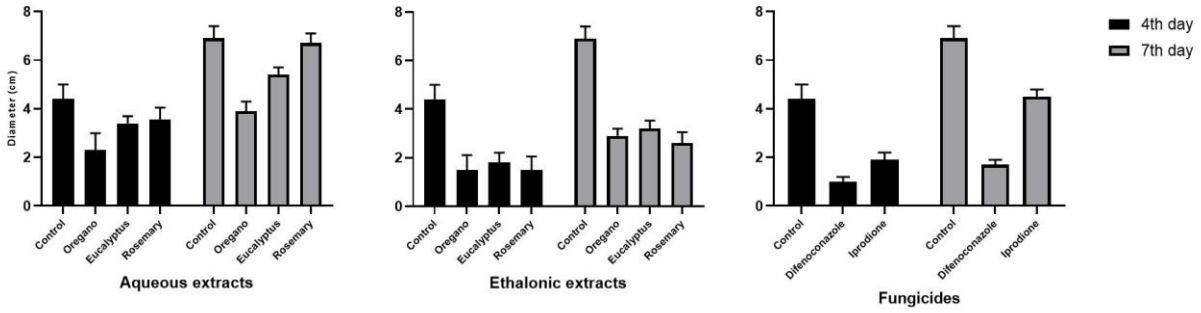

Figure 3. Effect of plants extracts and fungicides on radial growth of *F verticillioides*

In this study, we investigated the antifungal activity of aqueous and ethanolic extracts of three plants. The results showed that ethanolic extracts have a greater inhibitory effect than aqueous extracts. The percentage of inhibition of radial growth of *Fusarium verticillioides* did not exceed 50% with aqueous extracts. Similar findings were reported by Reklaoui et al. (2024); who studied the antifungal activity of aqueous extracts and essential oils of *Rosmarinus officinalis*, *Origanum compactum*, and *Origanum majorana* on ten phytopathogenic fungi, including four species of the genus *Fusarium*. They found that the inhibition rate of mycelial growth was equal to or less than 50% in the case of aqueous extracts.

The ethanolic extracts showed inhibition rates of 63.4 ± 2.4%, 57.7 ± 2.6%, and 65.2 ± 2.4% for oregano, eucalyptus, and rosemary, respectively. This can be attributed to the fact that ethanol is more effective than water in extracting certain secondary metabolites with antifungal properties, such as terpenes, carvacrol, and thymol in oregano; eucalyptol (1,8-cineole) in eucalyptus; and carnosol and camphor in rosemary (Chavan and Tupe, 2014; Coccimiglio et al., 2016; Lakušić et al., 2013; Mączka et al., 2023; Nakagawa et al., 2020; Sabo and Knezevic, 2019). The results obtained for the ethanolic extracts are superior to those of the fungicide iprodione (inhibition rate = 49.8%) but remain lower than those achieved with difenoconazole (inhibition rate=77.5%).

3.4. Effect of *A. muscarius, P. fluorescens* and *P. agglomerans* on radial growth of *F. verticillioides*

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The radial growth of *F. verticillioides* in the absence and presence of antagonistic organisms *(A. muscarius, P. fluorescens*, and *P. agglomerans)* is illustrated in Figure 4. The dual confrontation between *A. muscarius* and *F verticillioides* resulted in a reduction of colony diameter of *F. verticillioides* with an inhibition rate of 55 ± 3.3% between the 4th and 8th day of incubation. The strain of *P. fluorescens* was also effective in mycelium growth inhibition with a rate of 52.3 ± 5.1% in the same period. On the other hand, *P. agglomerans* was less effective than *A. muscarius* and *P. fluorescens.* The recorded inhibition rate was 44.7 ± 4%.

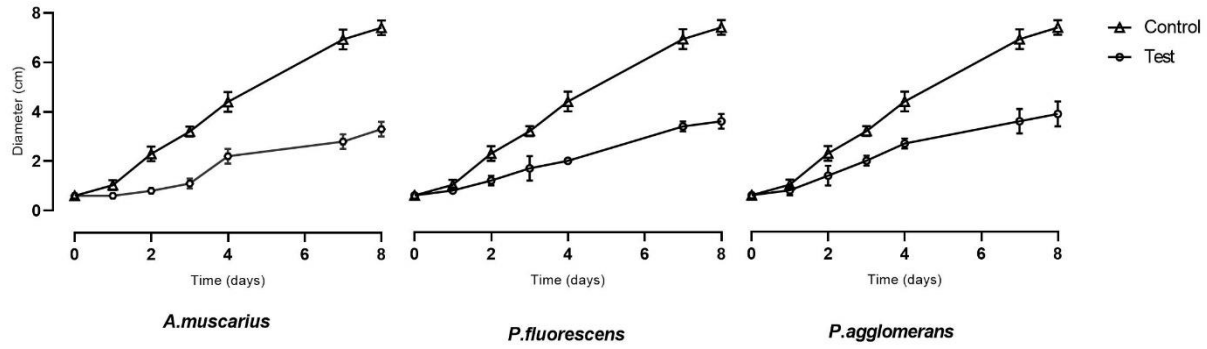

Figure 4. Effect of *A. muscarius*, *P. fluorescens* and *P. agglomerans* on radial growth of *F. verticillioides*

Direct confrontation tests using the entomopathogenic fungus *A. muscarius*, *P. fluorescens*, and *P. agglomerans* showed inhibition rates of 55%, 52%, and 44.7%, respectively. The results obtained with *A. muscarius* and *P. fluorescens* are superior to those recorded with the fungicide iprodione. Saidi et al. (2023), in similar tests, reported inhibition rates of *A. muscarius* against *Fusarium* species ranging between 39% and 52%. According to Turco et al. (2024) and Gomez-de La Cruz et al. (2022), this fungus exhibits antifungal activity against *Hemileia vastatrix*, the causal agent of coffee rust, as well as against the agents of powdery mildew in several plant species. The mechanisms involved include the production of chitinase and the destruction of spores. In the study by Mishra et al. (2022), *P. fluorescens* exhibited an inhibition rate of 69% against *F. verticillioides*. This inhibition was attributed to the synthesis of antifungal molecules such as 2,4-diacetylphloroglucinol. According to Xu et al. (2022), the *P. agglomerans* strain ZJU23 demonstrated efficacy in reducing the growth of *F. graminearum* through the production of Herbicolin A, which binds to and disrupts ergosterol.

3.5. Results of antagonism tests using wheat seeds

The inoculation of wheat seeds with *A. muscarius* at the same time as *F. verticillioides* caused an improvement in the germination rate, which increased from 4% to 22% in the case of durum wheat and from 3.1% to 25.3% in the case of bread wheat. This improvement in the germination rate was also accompanied by an improvement in the other two parameters (length and weight of coleoptile in germinated seeds). It should be noted that inoculation of grains with *A. mucarius* alone caused a slight decrease in the three parameters studied compared to those recorded in the control. In our study, the treatment of wheat grains inoculated with *F. verticillioides* using a suspension of *A. muscarius* moderately improved germination rates as well as the length and weight of the coleoptile. In other studies, treating wheat grains with entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium brunneum* ten days prior to inoculation with the phytopathogen *Fusarium culmorum* has been shown to effectively protect wheat plants. This protective effect is attributed to the colonization of both the roots and aerial parts of the plants by these entomopathogens (Jaber, 2018).

Table 1. Results of pathogenicity test and antagonism test using wheat seeds

	Treatment	Seeds germination rate (%)	Average coleoptile length (mm)	Average coleoptile weight (mg)
Durum wheat	Control	96.0 ± 1	23.3 ± 4.2	24.2 ± 4.9
	A. muscarius	92.0 ± 1	21.5 ± 4.1	20.44 ± 5.1

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	Treatment	Seeds germination rate (%)	Average coleoptile length (mm)	Average coleoptile weight (mg)
	F. verticillioides	4.0 ± 4	3.1 ± 1.78	1.46 ± .1.01
	F. verticillioides + *A. muscarius*	22.0 ± 6	7.68 ± 1.66	3.9 ± 2
Bread wheat	Control	97.0 ± 3	21.0 ± 2.1	20.3 ± 2.9
	A. muscarius	97.3 ± 3.5	18.53 ± 1.5	14.54 ± 2.1
	F. verticillioides	3.1 ± 2.8	2.5 ± 0.5	1.35 ± 0.6
	F. verticillioides + *A. muscarius*	25.33 ± 9.6	14.1 ± 1.7	11.1 ± 3

4.Conclusion

Isolate *S03 (F. verticillioides)* is pathogenic to wheat, causing a significant reduction in grain germination rates as well as decreases in coleoptile weight and length. The use of ethanolic extracts from oregano, eucalyptus, and rosemary, along with the fungus *A. muscarius* and the bacterium *P. fluorescens*, can inhibit the growth of this strain in vitro as effectively as fungicides. These alternatives may therefore represent an organic and less harmful solution. However, more extensive trials under field conditions are required before progressing to formulation trials.

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