Biocontrol potential of inflorescence rot of date palm caused by *Mauginiella scaettae* in the Biskra region (Algeria)

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Abstract

Date palm inflorescence rot (known as Khamedj disease) caused by *Mauginiella scaettae* is a serious problem in most date palm-growing areas of the world, and it causes considerable yield loss. The extensive use of fungicides has resulted in the emergence of fungicide-resistant pathogens, and concerns have been raised over the residual effects on the environment and human health. In this regard, biocontrol agents have been proposed as an alternative to standard fungicides. The aim of our study was to evaluate the biocontrol agent *Aspergillus niger* against the pathogen *M. scaettae*. *In vitro* confrontation tests between *M. scaettae* and *A. niger* showed that, after 10 days of incubation, the Petri dish was almost completely covered by the antagonist *A. niger*, while the pathogen *M. scaettae* occupied only 0.61±0.015 cm of diameter, which corresponds to a considerable inhibition of the mycelial growth (85.33%). Microscopic observations showed an abundant sporulation of *A. niger* around the colony of *M. scaettae* and marked a very important mycoparasitic power. In conclusion, the use of biological control agents is cost-effective, easy to use, and environmentally friendly for the control of Khamedj disease.

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Key words: antifungal activity, *Aspergillus niger*, biological control, *Mauginiella scaettae*, inflorescence rot of date palm.

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Introduction

Date palm inflorescence rot (also called "Khamedj" in North Africa) is one of the most important diseases affecting palms in many palm-growing areas of the world.[1,2] The disease affects both female and male inflorescences, especially in cold and fairly long winters accompanied by heavy rainfall.[3] The disease is mainly caused by the fungus *Mauginiella scaettae,*[4-6] which was first reported by Cavara in Libya in 1925.[2] In order to control the disease, various physical and/or chemical (fungicides) control methods have been adopted.[1,7,8] However, extensive use of fungicides has been raised over the residual effects and toxicity that affect the environment and human health.[9] Moreover, the efficacy of fungicides has decreased due to the emergence of resistant pathogens and implementation of new types of fungicides has become more difficult and more costly.[10]

Nowadays, biological control is becoming more and more essential and promising. This means of control is mainly based on the exploitation of the antagonism of certain fungal species such as *Trichoderma harzianum* and *Penicillium pinophilum*.[1,11,12] Indeed, no field experiments have been carried out on the use of biological control of palm inflorescence rot disease, and some antagonistic microorganisms are proposed to control this pathogen such as *Trichoderma harzianum, Penicillium pinophilum* and *Pseudomonas fluorescens*.[1] The present study is the first step in order to develop a biological control method based on antagonistic fungi against the inflorescence rot disease of date palms in Algeria. A great deal of attention has been paid to antagonistic microorganisms for reducing pathogen populations. *Aspergillus* spp. are important biological control agents due

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to their antagonistic activity, which depends on their different mechanisms of action during antagonism, specifically mycoparasitism by mycelial lysis and antibiosis by synthesis of volatile or non-volatile substances.[13] The antagonistic effect of *Aspergillus* species is due to its ability to produce a wide range of mycotoxins, including aflatrem, aflavinin, kojic acid, flavocol, aspergillic acid, aspertoxin, cyclopiazonic acid, paspallinin, and aflatoxins.[14] One of the *Aspergillus* species usually used for biocontrol is *Aspergillus niger*, which can produce mycotoxins such as ochratoxin, fumonisin, and aflatoxin.[15,16] Therefore, the aim of this study is to investigate, *in vitro*, the antifungal activities of the biocontrol agent *A. niger* against *M. scaettae*.

Materials and Methods

Isolation of pathogen

In order to isolate the pathogenic fungus, 25 infected spathes (18 male and 7 female spathes) were collected from palm groves in the Biskra region, South-East Algeria (34°51'00″ N and 5°44'00″ E) and then carried to the Mycology Laboratory of the National Institute of Plant Protection (INPV, Biskra) for cultivation. Small fragments (2 cm long) of infected date palm inflorescence were surface sterilized with 2% sodium hypochlorite for 3 minutes, rinsed three times for 3 minutes in sterile distilled water and blotted dry on sterilized filter paper. The fragments were then dried on sterile pads and placed on wet sterilized filter paper in Petri dishes, with 3 fragments per dish. Incubation took place at 25±2°C for 7 days in the dark. Once the colonies were well differentiated, they were re-inoculated several times in new Petri dishes containing Potato Dextrose Agar (PDA) medium to obtain pure cultures. After culturing, fungi have been identified by macro and microscopic observations based on the morphological characteristics of the mycelium and conidia.[2,17]

Antagonistic agents

The fungal antagonist *A. niger* was selected for testing against *M. scaettae in vitro* due to its strong antagonistic activity. The isolate of *A. niger* has been derived from the mycological collection of Dr. Dendouga Wassila (Department of Natural Sciences and Life, University of Biskra, Algeria).[18] The fungal cultures were kept on PDA medium at 25±2°C for further use (Figure 1).

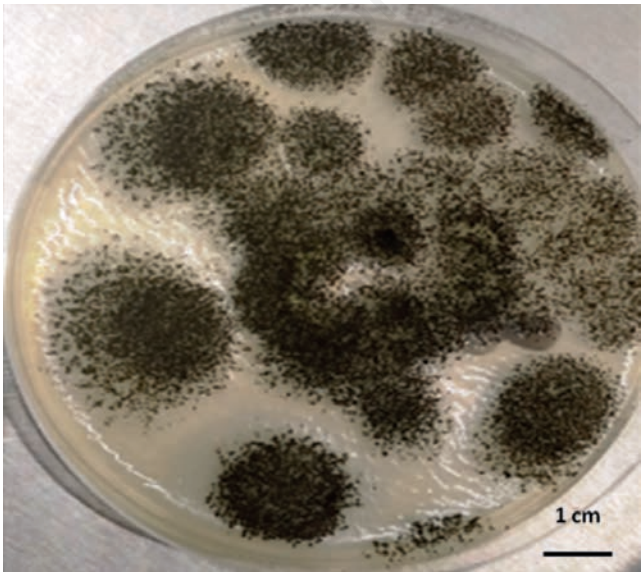

Figure 1. Pure culture of *A. niger.*

Evaluation of antagonistic activity

In order to evaluate the antagonistic activity of *A. niger,* the previously described technique was used.[19-21] For this, a 5 mm agar plug from 7 days old fungal cultures of pathogen and antagonist were placed into a Petri dish (9 cm diameter) containing 20 mL PDA. The explants were placed on an opposite diametrical axis 3 cm from each other and equidistant from the center of the plate. The plates were incubated in the dark at 25±2°C for 10 days. Control plates containing only the pathogen were incubated under the same conditions. Three replicates were performed for each test. Assessment of mycelial growth was carried out each day by measuring the average of two perpendicular diameters of the pathogen. The inhibition of mycelial growth of the pathogen by the antagonists was estimated by the following formula:

$$I\% = 1 - \frac{Cn}{Co} x100$$

Where:

I%: percentage of mycelial growth inhibition;
Cn: average colony diameter of the pathogen in the presence of the antagonist (cm);
Co: average diameter of colonies of the control (cm).

In addition, microscopic observations of the contact zone of the two isolates (pathogen-antagonist) were made in order to study the structural modifications affecting the *M. scaettae* isolates in antagonistic reaction to the *A. niger* strain. Three observations were made for each combination.[22]

Determination of fungicidal or fungistatic activity

In order to determine the nature of the effect of the antagonist fungi on the pathogenic fungi, we used the previously described method.[23] A 5 cm diameter disc of the pathogen aged 13 days from the start of the double culture was taken from the interaction zone (pathogen-antagonist) and placed in a Petri dish containing the PDA medium. Explants taken from a pure culture (containing only the pathogen) served as a control. The dishes were incubated at 25±2°C for 10 days. Three replicates were selected for each test. In the case of germination, the inhibitory action of the antagonist was assumed to be fungistatic, otherwise, it was fungicidal. The antagonist is considered fungistatic when the pathogen continues to grow during the additional incubation, and it is considered fungicidal in the absence of growth.

Statistical analysis

The results obtained were tested with statistical software (XLSTAT 2014.5.03). The means and standard deviation of three parallel measurements for each sample are represented. Significant differences between the means of mycelial growth values for control and confrontation (*M. scaettae* - *A. niger*) were analyzed by an independent two-tail t-test with $p<0.05$ as the level of significance.

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Results

Pathogen identification

The isolation was performed on 25 date palm spathes showing typical symptoms of inflorescence rot. The data obtained after isolation showed that *M. scaettae* was the causal agent of inflorescence rot. We have obtained pure cultures of *M. scaettae* from 24 spathes. The remaining spathe showed mixed infections with *Thielaviopsis* sp. and *Alternaria alternata.* After 7 days of incubation on PDA medium, the colonies of *M. scaettae* were white on the front and creamy to pale brown, becoming black in some isolates on the back. They reached 2.5 to 3 cm in diameter. Cottony appearance is characteristic of young colonies, which become powdery after abundant sporulation (Figure 2A). According to the results recorded, the mycelium is composed of branched hyaline and septate hyphae measuring between 15 and 80 µm long and 3 to 10 µm wide. Arthroconidia are unicellular or multicellular. Mature spores are unicellular, bicellular, tricellular, or multicellular (Figure 2B).

Direct confrontation between *M. scaettae* and *A. niger*

The obtained results indicate that the antagonist exerts a strong inhibitory power on the mycelial growth of *M. scaettae* where a percentage of 85.33% was recorded. Furthermore, our results show that the growth of *M. scaettae* was stopped already on the second day of confrontation (*M. scaettae* - *A. niger*) (Figure 3). After 10 days of incubation, the Petri dish was almost completely invaded by the antagonist, while the *M. scaettae* pathogen only occupied 0.61±0.015 cm in diameter compared to the control which reached 4.03±0.43 cm (Figure 4).

According to the t-test results, the antagonist *A. niger* had a highly significant effect on the pathogenic fungus *M. scaettae* (t=13.71, P=0.0001).

In the same context, the results showed that there was no zone of inhibition between the two fungi confronted and that the antagonist *A. niger* grew above and completely invaded the pathogen (Figure 5). On the other hand, the microscopic observations carried out showed abundant sporulation of *A. niger* around the colony of *M. scaettae* and marked a very important mycoparasitic power.

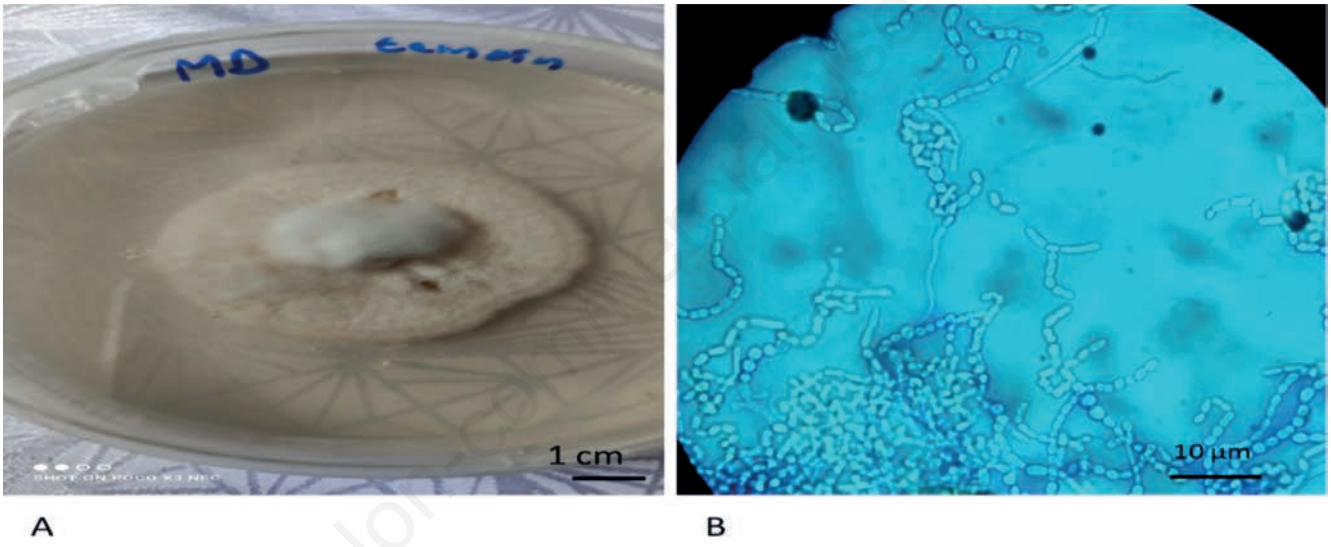

Figure 2. *Mauginiella scaettae* isolation from diseased date palm spathes. A) the fungus' development on PDA; B) the hyphae and conidia of the fungus.

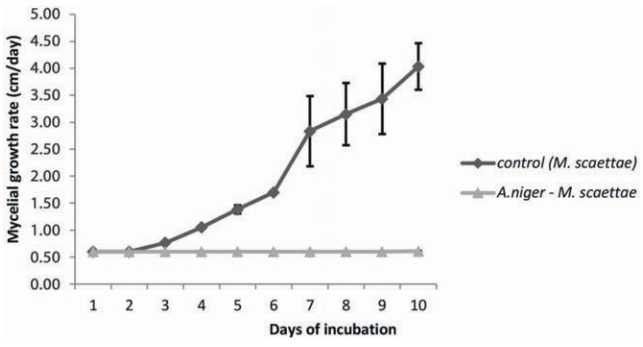

Figure 3. Evolution of mycelial growth of *M. scaettae* in direct confrontation against *A. niger* during 10 days of incubation.

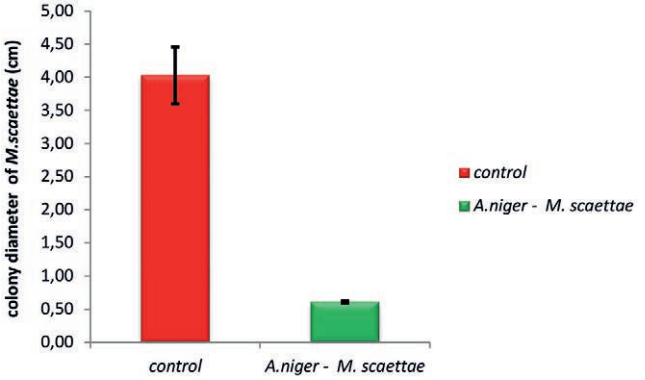

Figure 4. Colony diameter of *M. scaettae* after 10 days of incubation

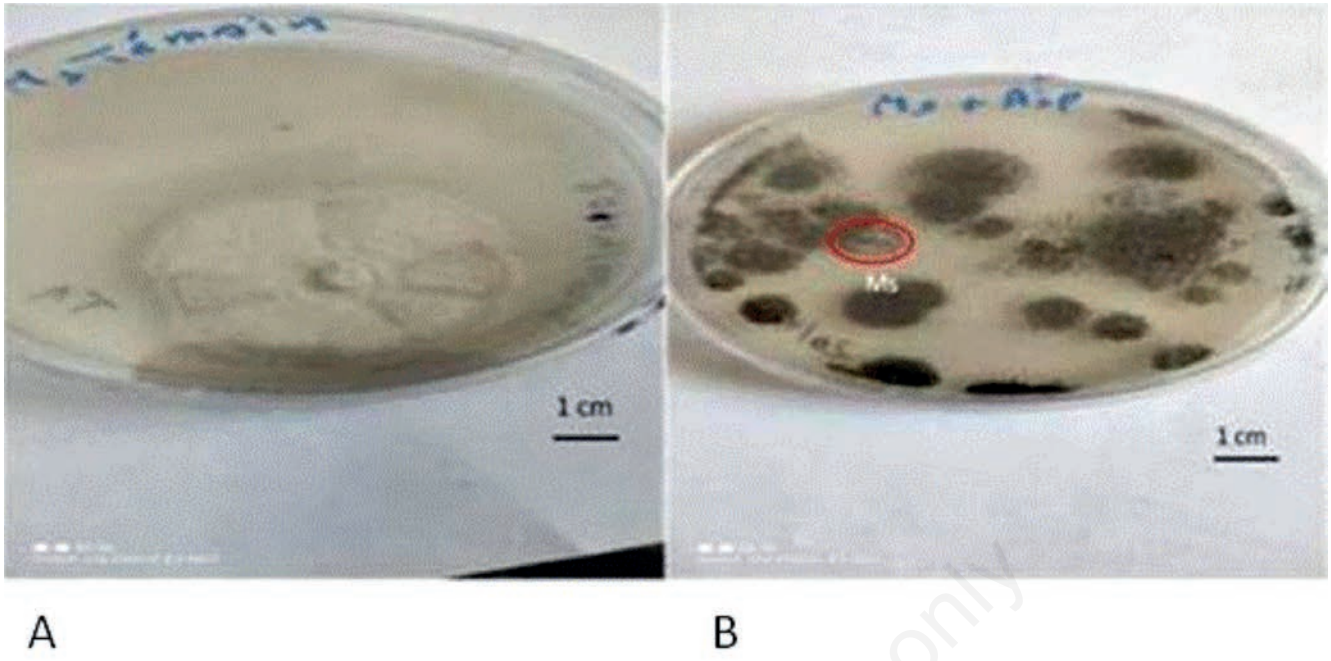

Figure 5. Direct confrontation between *M. scaettae* and *A. niger.* A) colony of *M. scaettae* (control); B) direct confrontation *M. scaettae* - *A. niger.*

Fungicidal or fungistatic action

According to the obtained results, it was noted that the tested antagonist fungus showed a strong inhibition against the fungal strains of *M. scaettae*. In view of this complete inhibition of the pathogenic strains, it was important to know whether the antagonist was fungistatic or fungicidal. Indeed, the transfer of the totally inhibited pathogenic fungus discs to another PDA culture medium was necessary to investigate the viability of these fungi.

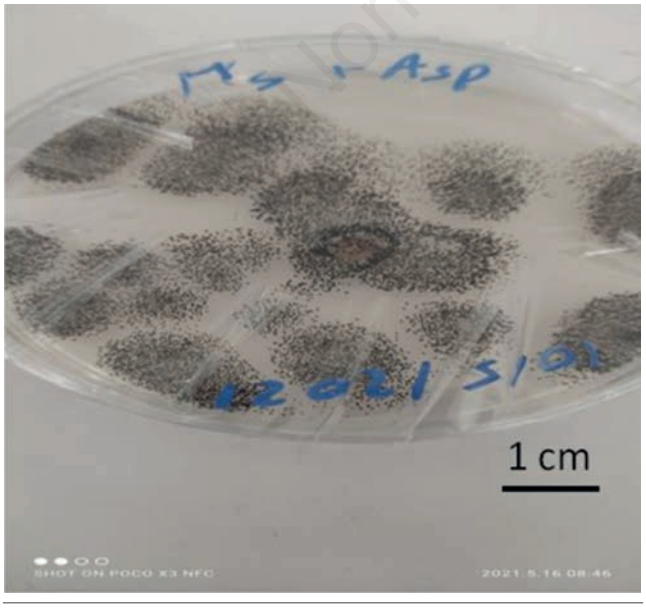

Figure 6. Fungicidal effect of *A. niger* against *M. scaettae.*

The results obtained showed that the *M. scaettae* fungus did not grow and that mycelial growth did not reappear after the transfer of the disc, indicating that the fungal antagonist effect tested was fungicidal and that the pathogenic fungus was completely destructed (Figure 6).

Discussion

In this study, we identified *M. scaettae* as the causal agent of inflorescence rot disease. Our results are consistent with those of other studies, according to which *M. scaettae* causes inflorescence rot.[2,5,24,25] Nonetheless, other fungi, including *Fusarium oxysporum, F. moniliforme, F. solani, Trichothecium roseum, Botrytis aclada, Thielaviopsis paradoxa, Acremonium strictum,* and *Memmoniella* sp., have been reported in multiple cases of date palm inflorescence rot.[2,17,25-28]

Fungal diseases are usually treated with chemicals, which have harmful and toxic effects on human health and the environment. It is therefore essential to develop alternative solutions to control these diseases. Indeed, pathogens can be controlled biologically using other antagonistic microorganisms that can totally or partially destroy the pathogens.[29] This study was conducted with the prospect of developing a biocontrol-based management strategy against *M. scaettae*, the main causative agent of date palm inflorescence rot. Specifically, the use of biocontrol agents derived from such a wide range of natural materials as viruses, bacteria, fungi, nematodes, and plant extracts have been reported to hold great potential in alleviating plant disease stress. The present study showed that the tested antagonist *A. niger* significantly inhibited the growth of the pathogen *M. scaettae*. On the other hand, it was reported that isolated *A. niger* reduced the diametrical growth of *Pythium* sp. colonies (inhibition rates 19.94-24.12%).[30] Furthermore, the *in vitro* and *in vivo* effect of the interaction between *A. niger*, *Mucor* sp. *Fusarium oxysporum*, *Fusarium*

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solani, *Phoma* sp., *Penicillium* sp., and *Trichoderma* sp., showed that *A. niger* inhibited the growth of *Fusarium oxysporum* by 92.30%.[31] Hence, *A. niger* had a severe inhibitory effect against *M scaettae*. In this case, the direct contact between the antagonist and the pathogen revealed that there was no antibiosis zone between them, and that the antagonist was growing above the pathogen, indicating that antagonism can occur through the mechanism of fungal parasitism. Parasitism is manifested by the destruction of the pathogen when *A. niger* wraps itself around the pathogen either by strangling it, penetrating its interior and/or "injecting" substances (enzymes) that destroy it. Indeed, antifungal biomolecules are secondary metabolites produced by antagonists during antibiosis, such as antibiotics and toxins, and/or during mycoparasitism, such as lytic enzymes. These biomolecules have antifungal activity against phytopathogenic fungi.[30, 32]

Conclusions

In light of these investigations carried out *in vitro*, it can be concluded that the antagonist *A. niger* has reducing properties on mycelial growth and sporulation of *M. scaettae*. Due to their fungicidal activity, this antagonistic fungus could be an effective biological control agent capable of reducing the severity of date palm inflorescence rot and used as an alternative in integrated biological control systems against pathogenic microorganisms. In addition, the application is cost-effective and environmentally safe.

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