Evaluation of the in vitro antagonistic effect of fungal species extracted from the argan tree, for biological control of pathogenic colonies

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ABSTRACT

The argan tree, the emblematic tree of Morocco, is of crucial importance in today's food security context. Thriving in arid and semi-arid regions, this tree plays an essential role both ecologically and economically, being the exclusive source of argan oil prized in many fields, including cuisine and cosmetics. However, despite its resilience, the argan tree faces its fair share of challenges, such as phytopathogens synthesizing toxic metabo-lites that compromise harvest quality. Our study focuses on the in vitro antagonistic action of purified strains of *Mucor spp*., *Aspergillus Niger* and *Penicillium spp*., extracted from argan fruits, against pathogens such as *Fusarium spp*. and *Cladosporium spp.* The evaluation of the pathogenic strains' antagonistic power was carried out by indirect confrontation at a constant temperature of 25°C over five days. The results obtained show significant inhibition of pathogen growth, and underline the strong potential of these antagonistic agents in biological control for sustainable agriculture and environmentally-friendly farming practices.

Keywords
biological control ; antagonist ; inhibition ; pathogens

RÉSUMÉ

Évaluation de l'effet antagoniste in vitro des espèces fongiques extraites de l'arbre d'argan, pour le contrôle biologique des colonies pathogènes

L'arbre d'argan, emblème du Maroc, revêt une importance cruciale dans le contexte actuel de la sécurité alimentaire. Poussant dans des régions arides et semi-arides, cet arbre joue un rôle essentiel à la fois écologique et économique, étant la source exclusive de l'huile d'argan, prisée dans de nombreux domaines, notamment la cuisine et la cosmétique. Cependant, malgré sa résilience, l'arbre d'argan fait face à des défis, tels que des phytopathogènes synthétisant des métabolites toxiques compromettant la qualité des récoltes. Notre étude porte sur l'action antagoniste in vitro de souches purifiées de *Mucor spp*., *Aspergillus niger* et *Penicillium spp*., extraites des fruits de l'arganier, contre des pathogènes tels que *Fusarium spp*. et *Cladosporium spp*.. L'évaluation du pouvoir antagoniste des souches pathogènes a été réalisée par confrontation indirecte à une température constante de 25°C sur une durée de cinq jours.

Mots clés
lutte biologique ; antagoniste ; inhibition ; pathogènes

Les résultats obtenus montrent une inhibition significative de la croissance des pathogènes et soulignent le fort potentiel de ces agents antagonistes dans la lutte biologique pour une agriculture durable et respectueuse de l'environnement.

1. INTRODUCTION

The argan tree, an endemic Moroccan tree, takes on a special dimension in today's context of food productivity issues. Extending over some 871,210 hectares nationwide and thriving in the country's arid regions, the argan tree plays a vital role both ecologically and in the local economy, making a significant contribution to combating desertification and soil erosion while fostering a specific biodiversity [1].

At the very heart of the Moroccan argan grove, this resilient tree provides an essential habitat for numerous species and is the exclusive source of argan oil, a renowned product whose multiple functions have long shaped and influenced the lives of local populations, and whose immense potential has aroused the particular interest of scientists.

However, despite its remarkable robustness, the argan tree is not without its challenges. Demographic change, variable climatic conditions and growing consumer habits have led to the development of a range of plant pathogens affecting argan crops. These pathogens, extracted from argan fruits, are the cause of many devastating diseases and toxic sec-ondary metabolites, affecting the crop's sanitary quality.

The widespread use of chemical pesticides, once considered the main solution against pathogens, is now under question due to its negative impact on ecosystems and human health, as well as the resistance developed by pathogens. Agricultural production is therefore faced with a number of constraints: on one hand, climatic changes that are conducive to microbial development and toxin production, and on the other hand, in-creasingly stringent regulations to limit the use of phytosanitary products.

In light of these constraints, biological control is emerging as a promising alternative. The aim of our study is to evaluate the indirect in vitro antagonistic action of purified strains of Mucor spp., Aspergillus Niger and Penicillium spp., extracted from argan fruits, against pathogens such as Fusarium spp. and Cladosporium spp.

This innovative approach is part of the long-term goal of developing sustainable solutions to counter the pathogenic threats to argan crops, thereby preserving the delicate balance of the Moroccan argan grove.

2. MATERIALS AND METHODS

2.1. Biological material

In this study, the phytopathogens used, namely Cladosporium spp., Fusarium spp., Mucor spp., Penicillium spp. and Aspergillus Niger, came from the mycological laboratory col-lection at the 'Institut agronomique et vétérinaire Hassan II'. They were isolated from argan fruits originating from Essaouira.

2.2. Culture medium

The culture medium used in this study is PDA (Potato Dextrose Agar), widely used for the cultivation, isolation and purification of fungi. The evaluation of indirect interactions between the purified strains will be carried out in vitro on this medium, for 5 days.

Before use, the culture medium is sterilized in an autoclave for 20 minutes at 121°C. Still warm (at around 50°C), it is aseptically divided into sterile Petri dishes (15-20 ml each). Once the agar has solidified, the plates are ready for use [2].

2.3. Purification and conservation

In order to isolate a single colony per Petri dish, subculturing is performed. The process, carried out aseptically near a Bunsen burner, involves removing a colony disk with a sterilised inocultating loop and placing it in the center of a new dish containing the same PDA culture medium, by avoiding any contact with nearby colonies [3].

The plates are then incubated at 30°C for seven days to obtain pure strains. This process is repeated as many times as necessary, until totally pure isolates are obtained.

To preserve them, the strains obtained are transferred

to Agar slant vials, kept in an incubator at 25°C for 6 to 7 days, then stored at 4°C for later use.

2.4. Fungal antagonism test

To realize the fungal antagonism test, the antagonist and the studied pathogen are in-oculated into 2 distinct PDA Petri dishes, following the indirect co-culture method by (Abdallah et al., 2018) [4]. The 2 Petri dishes are then placed against each other in a face-to-face manner, with the antagonist dish at the bottom and the pathogen at the top ; using layers of parafilm, the junction between the two plates is sealed to prevent any loss of volatile substances [5].

A control is established by inoculating the pathogen in the center of a Petri dish, and superposing it to one containing only the PDA medium. All the prepared Petri dishes are then incubated for 5 days at a temperature of (25 ± 2°C).

Results were assessed daily during the 5-day incubation period by measuring the di-ameter of the mycelial growth zone. The inhibition percentage of mycelial growth was calculated using the following formula:

$$In(\%) = \frac{(C_0 - C_n)}{C_0} \times 100 \qquad [5]$$

With : C_0 : The diameter of pathogenic colonies in the control ; Cn : The diameter of pathogenic colonies in the presence of antagonists.

The chosen antagonist/pathogen combinations are as follows:

- *Penicillium spp.* (antagonist) and *Cladosporium spp.* (pathogen);
- *Aspergillus Niger* (antagonist) and *Fusarium spp. (pathogen)* ;
- *Mucor spp*. (antagonist) and *Cladosporium spp*. (pathogen).

3. RESULTS

3.1. Inhibitory effect of Penicillium spp. on Cladosporium spp.

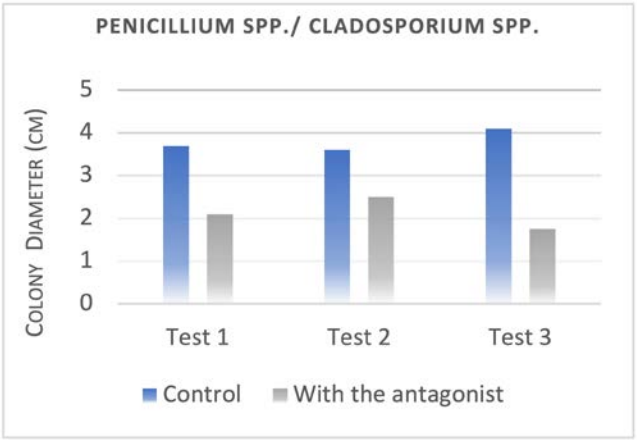


Figure 1. Diameter of Cladosporium spp. colonies in indirect confrontation with Penicillium spp. after a 5-day incubation at 25°C.

The inhibitory effect of Penicillium spp. can be seen in the significant reduction in *Cladosporium spp.* colony diameter, averaging around 45%.

3.2.Inhibitory effect of Aspergillus Niger on Fusarium spp.

The results obtained show a clear reduction in

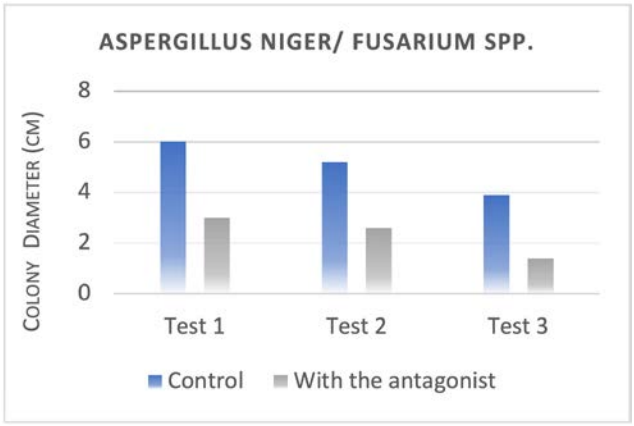


Figure 2. Diameter of Aspergillus Niger colonies in indirect confrontation with Fusarium spp. after a 5-day incubation at 25°C

the diameter of Fusarium spp. colonies in the presence of Aspergillus Niger compared with the untreated control. This reduction translates into a very significant mycelial growth inhibition rate of around 60%.

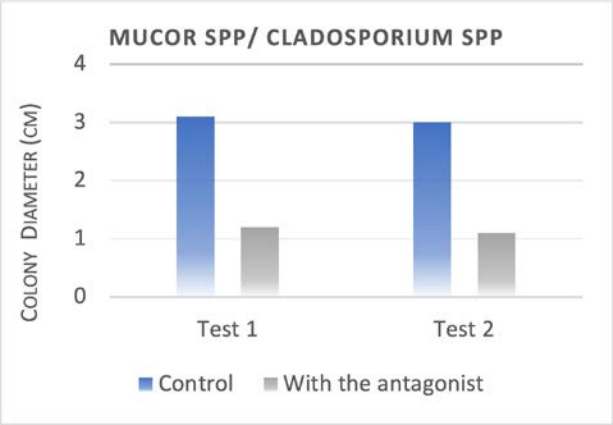


Figure 3. Diameter of Mucor spp. colonies in indirect confrontation with Cladosporium spp. after a 5-day incubation at 25°C.

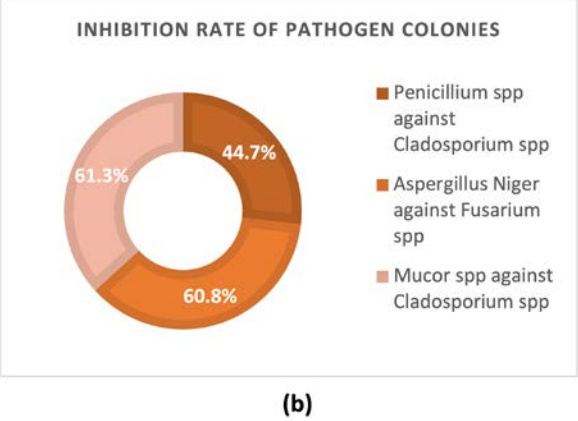
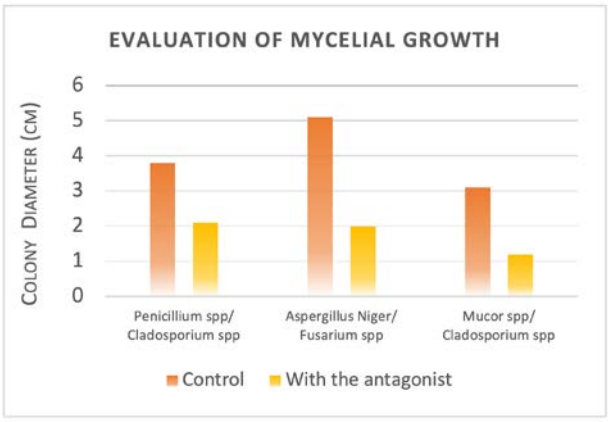


Figure 4. (a) Antagonist colonies' long-distance inhibitory effect on pathogens; (b) Inhibition rate of pathogenic colonies in indirect confrontation with antagonistic agents

After a 5-day incubation period, the Petri dish was completely invaded by the antagonist (Mucor spp.), at a remarkably rapid rate. Cladosporium spp. underwent a mycelial growth inhibition rate of over 60%, dropping from 3.1cm diameter (in the control), to 1.2 on average in the presence of the antagonist.

3.4. Evaluation of mycelial growth

To evaluate the mycelial growth of the pathogenic strains studied, we measured the average diameter of pathogenic colonies after a 5-day incubation period at 25°C, with and without the presence of antagonists.



Despite the absence of direct contact between the pathogenic strains tested, Fusarium spp. and Cladosporium spp., against the antagonistic agents : Aspergillus Niger, Mucor spp. and Penicillium spp., the latter were able to exert inhibitory activity on the mycelial development of pathogenic colonies at significant inhibition rates. This can be explained by the antago-nists' ability to produce volatile substances that inhibit the pathogen's mycelial growth.

4. DISCUSSION

Despite the absence of direct contact between the pathogenic strains tested, Fusarium spp. and Cladosporium spp., against the antagonistic agents : Aspergillus Niger, Mucor spp. and Penicillium spp., the latter were able to exert inhibitory activity on the mycelial devel-opment of pathogenic colonies at significant inhibition rates. The observed outcomes include a 61.3% inhibition of Cladosporium spp. by Mucor spp., a substantial 60.8% inhi-bition of Fusarium spp. by Aspergillus Niger, and a commendable 44.7% inhibition of Cladosporium spp. by Penicillium spp. This can be explained by the antagonists' ability to produce volatile substances that inhibit the pathogen's mycelial growth.

It's also worthy to note that within the realm of fungi, Chitin stands as a crucial constituent of the cell wall, serving to envelop and shield fungal cells from the external environment. Consequently, the cell wall plays a pivotal role in both fungal development and

resilience against external threats. Any modification, such as that linked to the virulence of the inoculum, would result in an impairment of the mycelium [6]. This underscores the potent myco-parasitic capabilities exhibited by Aspergillus Niger, Mucor spp, and Penicillium spp.

Additionally, the substantial lysis of mycelium, as mentioned in other studies, could also be a contributing factor to the inhibition of the mycelial growth in Fusarium spp. and Cladosporium spp. [7].

Our findings emphasize the great potential of these antagonistic agents in biologically controlling crop pathogens and suggest potential applications in crop protection.

As a matter of fact, exploiting them presents an opportunity to develop more sustainable agricultural strategies, reducing reliance on chemical pesticides while safeguarding crop health. This study thus makes a meaningful contribution to the promotion of environ-mentally friendly agricultural practices that prioritize the well-being of plants.

5. CONCLUSION

The results obtained during this study revealed an interesting inhibitory effect of argan fruit extracts on the growth of pathogenic microorganisms such as Fusarium spp. and Cladosporium spp. This paves the way for potential applications in the field of crop pro-tection. By exploiting these biological control agents, it is possible to develop more sus-tainable agricultural strategies, limiting the dependence on chemical pesticides while preserving the crop's sanitary quality. Our next step would be to deepen our research, assessing the inhibitory effect of other isolates, before undertaking our in vivo studies.

REFERENCES

1. Aziz, L., Mormont, M., & Allali, K. (2013). Effets de la marchandisation de l'arganier sur la vie socioéconomique des populations de l'arganeraie marocaine. VertigO, (Volume 13 Numéro 1). DOI:10.4000/vertigo.13445

2. Udawattha, C., Galkanda, H., Ariyarathne, I. S., Jayasinghe, G. Y., & Halwatura, R. (2018). Mold growth and moss growth on tropical walls. Building and Environment, 137, 268–279. DOI:10.1016/j.buildenv.2018.04.018

3. Guiraud J.P. (2003). Microbiologie alimentaires. (edn) Dunod. Paris.p : 651.

4. F Abdallah, M., De Boevre, M., Landschoot, S., De Saeger, S., Haesaert, G., & Audenaert, K. (2018). Fungal Endophytes Con-trol Fusarium graminearum and Reduce Trichothecenes and Zearalenone in Maize. Toxins, 10(12), 493. Doi: 10.3390/toxins10120493

5. Tian, Y., Yu, D., Liu, N., Tang, Y., Yan, Z., & Wu, A. (2020). Confrontation assays and mycotoxin treatement reveal antagonistic activities of Trichoderma and the fate of Fusarium mycotoxins in microbial interaction. Environmental Pollution, 267, 11559. DOI: 10.1016/j.envpol.2020.115559

6. Benhamou, N., & Chet, L. (1996). Parasitism of sclerotia of Sclerotium rolsii by Trichoderma harzianum:ultrastructural and cytochemical aspects of the interaction. Phytopathology, 86, 405-4160.

7. Daami-remadi, M., & El Mahjoub, M. (2001). Lutte biologique contre la pourriture aqueuse des tubercules de pomme de terre par Trichoderma harzianum. Ann.L'INRAT, 74, 167-186.