Rev Iberoam Micol. 2016;33(2):129–130

Revista Iberoamericana de Micología

www.elsevier.es/reviberoammicol

Letter to the Editor

Assessment of compatibility between the nematophagous fungi *Arthrobotrys robusta* and *Duddingtonia flagrans* under laboratory conditions

Valoración de la compatibilidad entre los hongos nematófagos Arthrobotrys robusta *y* Duddingtonia flagrans *en condiciones de laboratorio*

Nematophagous fungi are used in the biological control of pests and diseases.[1,2,5,7,10] The use of more than a single biocontrol agent is considered one of the main suppressive measures contributing to control the presence of infectious agents in soils.[8] The use of more than one nematophagous fungi in combination can minimize possible flaws in their administration or even enhance their actions as biocontrol agents.[6] However, biological control mechanisms such as the capability to produce substances having a fungicidal effect, may vary among species and even among isolates of the same species during the life of the antagonist.[9]

This work aimed to study the compatibility between *Arthrobotrys robusta* and *Duddingtonia flagrans* under laboratory conditions by means of direct confrontation, antibiosis and volatile metabolites tests.

Two predatory fungi, *D. flagrans* (AC001) and *A. robusta* (I31) were used. A culture disc containing AC001 and a disc with I31 were placed on opposing sides of a Petri plate in culture medium containing 2% potato dextrose agar. These plates were incubated for 10 days at 26 °C in the dark and, after this period, were evaluated with a rating scale.[3] The experiment had five repetitions.

The antibiosis test followed the modified methodology of Martins-Corder and Melo.[9] The plates were then kept at 26 °C in the dark. After 10 days of incubation, we assessed the formation of inhibition zones.[9]

The effect of volatile metabolites was evaluated with Bharat et al.[4] methodology. The plates were sealed laterally and maintained at 25 °C for five days in the dark. The area of each colony was measured and then the data were submitted to ANOVA and Tukey's test at significance levels of 1% and 5%, and the degrees of freedom of the treatments deployed in orthogonal contrasts. The experiment, in a completely randomized design, had four replications.

Two aliquots of the same species of fungus grown in the same plate showed homogeneous growth in a direct confrontation test, confirming the non-interference of one colony on the other (Fig. 1A and C). However, in the test with *A. robusta* and *D. flagrans*, I31 colonized approximately 2/3 of the plate, suggesting competition between them and therefore some antagonism (Fig. 1B). However, there was no evidence of hyper parasitism without growth of a colony on each other.

The production of toxic metabolites by *D. flagrans* and *A. robusta* is unknown; therefore, it was important to show that they do not produce compounds capable of inhibiting one another in a joint application. This was verified by an absence of antibiotic production by both fungi in the culture medium, as a result of which there were no zones of inhibition when a culture was subsequently sown on the same plate. Nevertheless, *A. robusta* reduced the growth of *D. flagrans*, suggesting the action of volatile antibiotics in inhibiting mycelial growth. Furthermore, *D. flagrans* did not reduce the growth of *A. robusta* (Table 1).

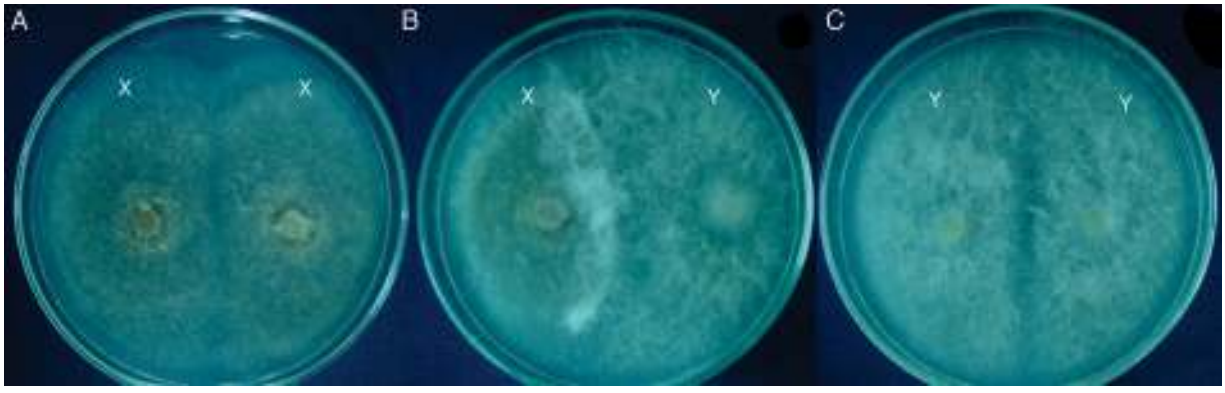

Fig. 1. Growth together of colonies of *Duddingtonia flagrans* (X) and *Arthrobotrys robusta* (Y). (A) *D. flagrans* vs. *D. flagrans*; (B) *D. flagrans* vs. *A. robusta*; (C) *A. robusta* vs. *A. robusta*.

http://dx.doi.org/10.1016/j.riam.2015.07.001

Table 1
Mean values and standard deviation (±) of the area (cm^2) of mycelial growth in covers of Petri dishes by the fungi *Duddingtonia flagrans* (top cover) vs. *Arthrobotrys robusta* (lower cover) and vice versa when compared to *D. flagrans* vs. *D. flagrans* and *A. robusta* vs. *A. robusta* respectively.

Duddingtonia flagrans (top lid)		*Arthrobotrys robusta* (top lid)	
Treatments	Area (cm^2)	Treatments	Area (cm^2)
D. flagrans vs. *A. robusta*	$28.50^{*} \pm 6.01$	*A. robusta* vs. *D. flagrans*	$57.61^{ns} \pm 1.10$
D. flagrans vs. *D. flagrans*	34.98 ± 0.52	*A. robusta* vs. *A. robusta*	57.06 ± 1.28
CV (%)	21.09		1.91

CV: coefficient of variation.
ns: not significant by Tukey test at 5% probability.
* Significant at 5% probability.

Author's declaration

We declare that: (a) the content of the article is original and was not published previously; (b) there is no conflict of interests, related to financial aspects; (c) all the authors have read and approved this manuscript.

Acknowledgments

We thank the Universidade Federal de Viçosa for its professionalism in developing education, research and extension, and also the FAPEMIG and CNPq for the financial support.

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