

Introduction

- Fungal co-culture has received limited attention for use in enhancing fungal biomass fitness and for harnessing the unique beneficial traits of different species.
- Penicillium* sp. (F1) and *Trichoderma* sp. (F2) strains were identified to have valuable plant growth promotion and plant pathogen suppression attributes.
- In this study, a co-culture (Mix) of F1 and F2 was grown and assessed for its ability to inhibit the growth of four plant pathogens *in vitro*, reduce disease severity *in planta*, and tolerate UV-C irradiation.

Aim

Assess the effect of fungal co-culture on biocontrol activity and photostability of the consortium of *Penicillium* sp. and *Trichoderma* sp.

Methodology

- In vitro* competition assays:**

Treatments (F1, F2 and mix) and each fungal pathogen were inoculated on opposite sides of agar plates in triplicate. The colony diameters of pathogens were measured on days 3, 5, and 7 after inoculation (Figure 1A).
- In planta* bioassay / biocontrol activity:**

Ten fragments of tomato stems (10 cm) vertically inserted in a piece of wet oasis foam were inoculated on the top with the treatments (F1, F2 and Mix). One day later, the pathogen *Botrytis cinerea* was inoculated and the development of the disease was scored after 7 days of incubation in humid chambers.
- Photostability test:**

Serial dilutions of treatments (F1, F2 and Mix) were inoculated in triplicate, exposed to 1-minute or 5-minutes of UV-C and incubated for 7 days (Figure 1B). Colonies were counted thereafter.

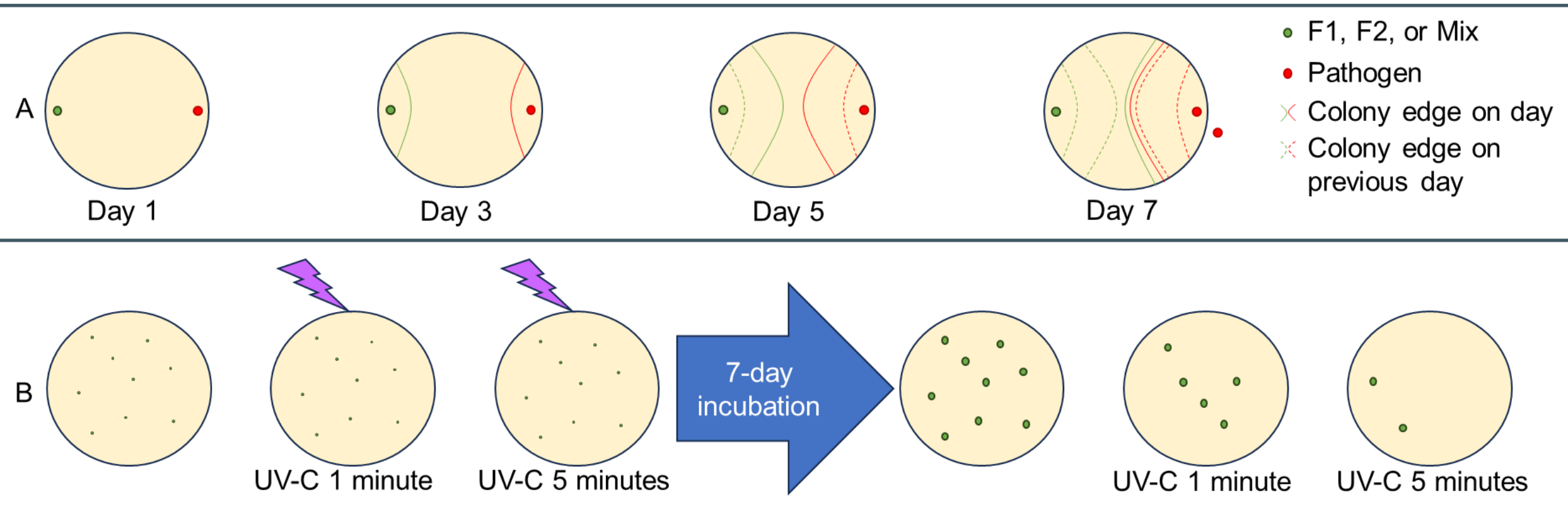


Figure 1. Methodologies for *in vitro* competition bioassay (A), and UV-C exposure experiment (B)

Results

- In vitro* competition assays:**

The Mix and axenic F2 cultures were equally efficient at inhibiting the diametral growth of four plant pathogens (*Fusarium oxysporum* (Figure 2), *Rhizoctonia solani*, *Botrytis cinerea*, and *Sclerotinia sclerotiorum*) (Figure 3).

References

1. Goldman, G. H., McGuire, S. L., & Harris, S. D. (2002). The DNA damage response in filamentous fungi. *Fungal Genetics and Biology*, 35(3), 183-195.
2. Boughalleb-M'Hamdi, N., Salem, I. B., & M'Hamdi, M. (2018). Evaluation of the efficiency of *Trichoderma*, *Penicillium*, and *Aspergillus* species as biological control agents against four soil-borne fungi of melon and watermelon. *Egyptian Journal of Biological Pest Control*, 28, 1-12.

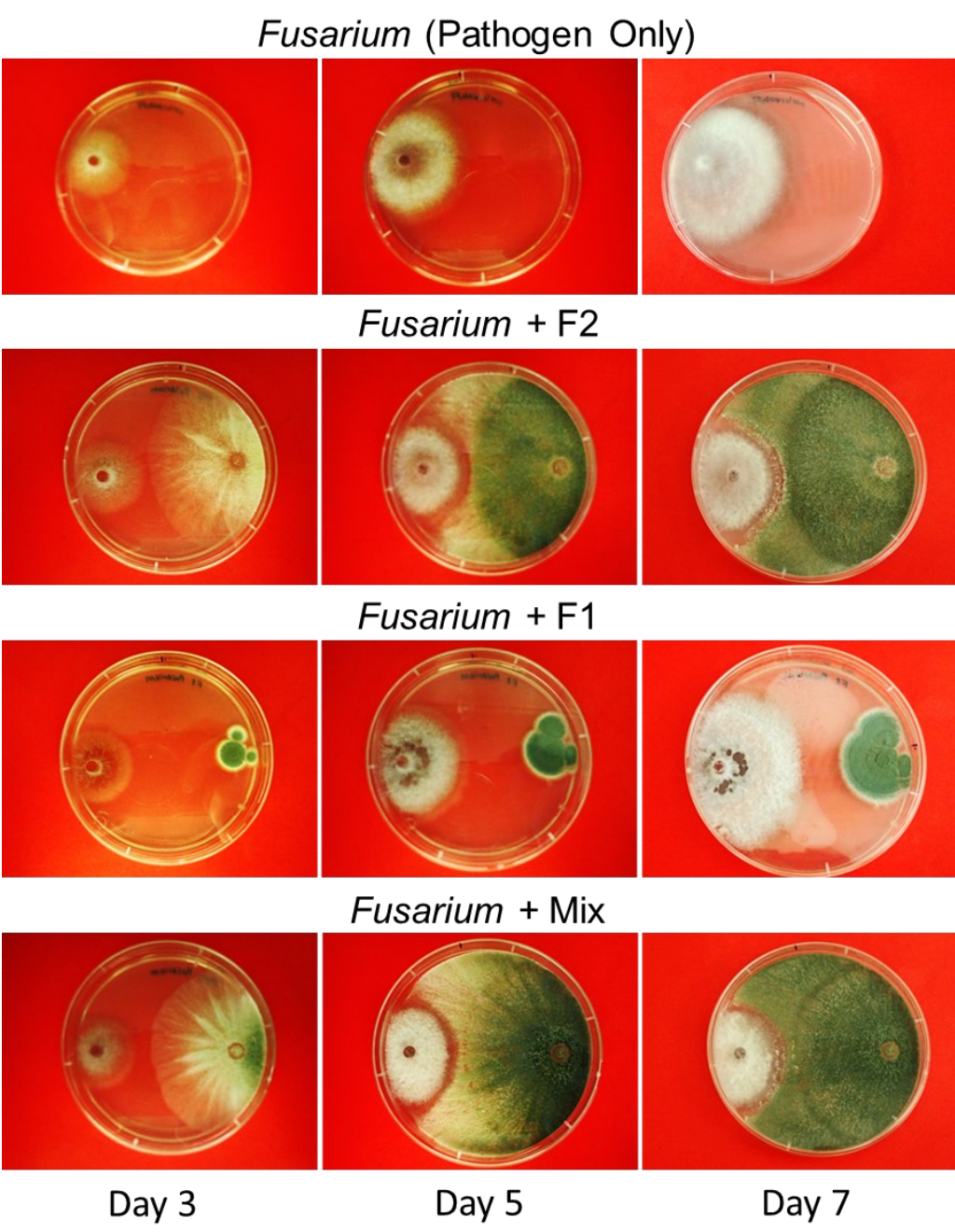


Figure 2. *In vitro* competition assay
The fungus on the left of each plate is the plant pathogen *F. oxysporum* and fungi on the

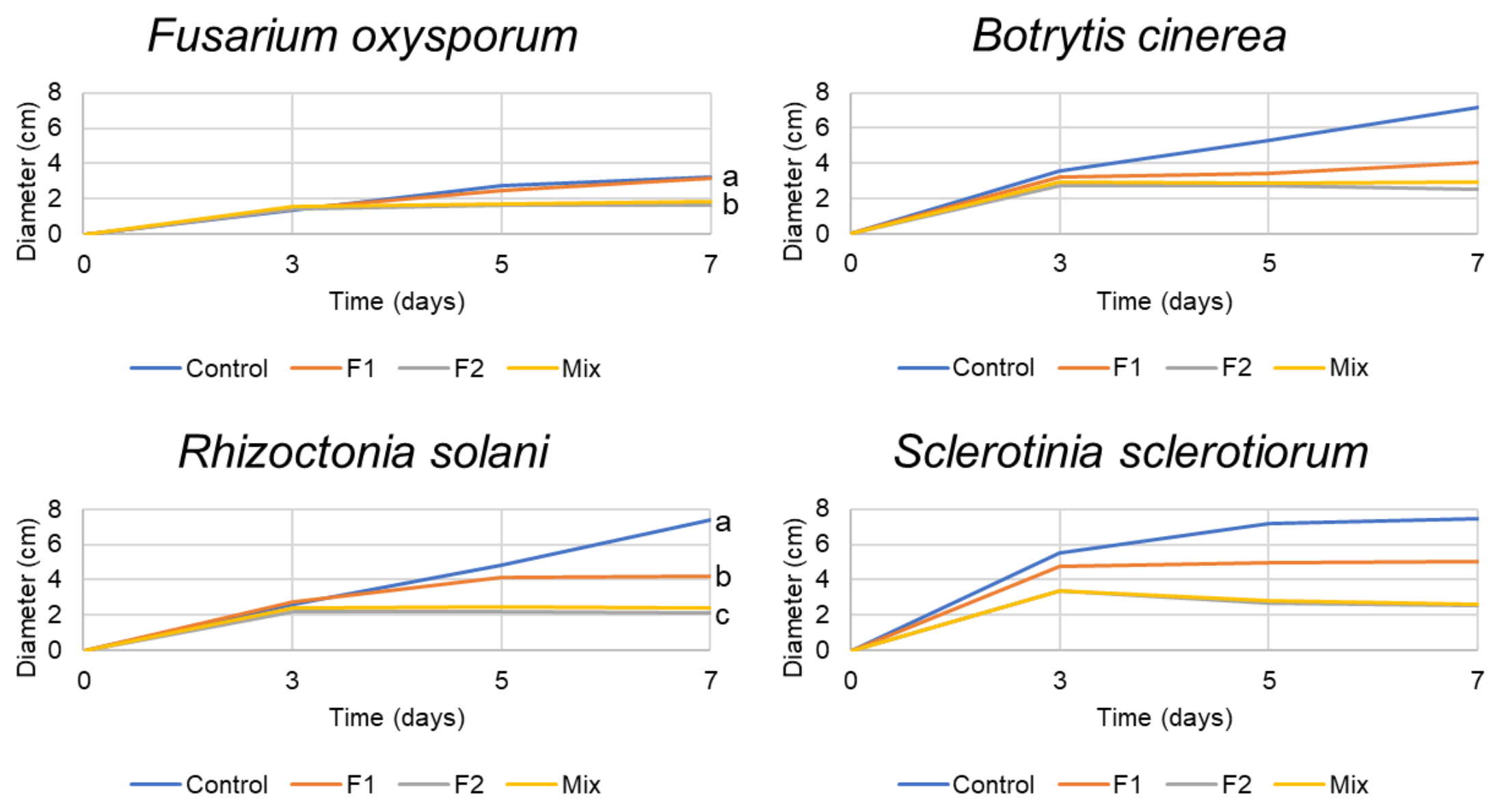


Figure 3. Effect of co-cultured beneficial fungi (Mix) on diametral growth of plant pathogens
Treatments with the same letter are not significantly different according with Tukey test (95%)

- Effect of co-culture on biocontrol activity:**

There was a significantly higher ($P<0.05$) reduction in disease severity in tomato stems inoculated with the Mix culture (severity reduced by 60%) than the reduction caused by axenic F2 (28%) and F1 (41%) (Figure 4).

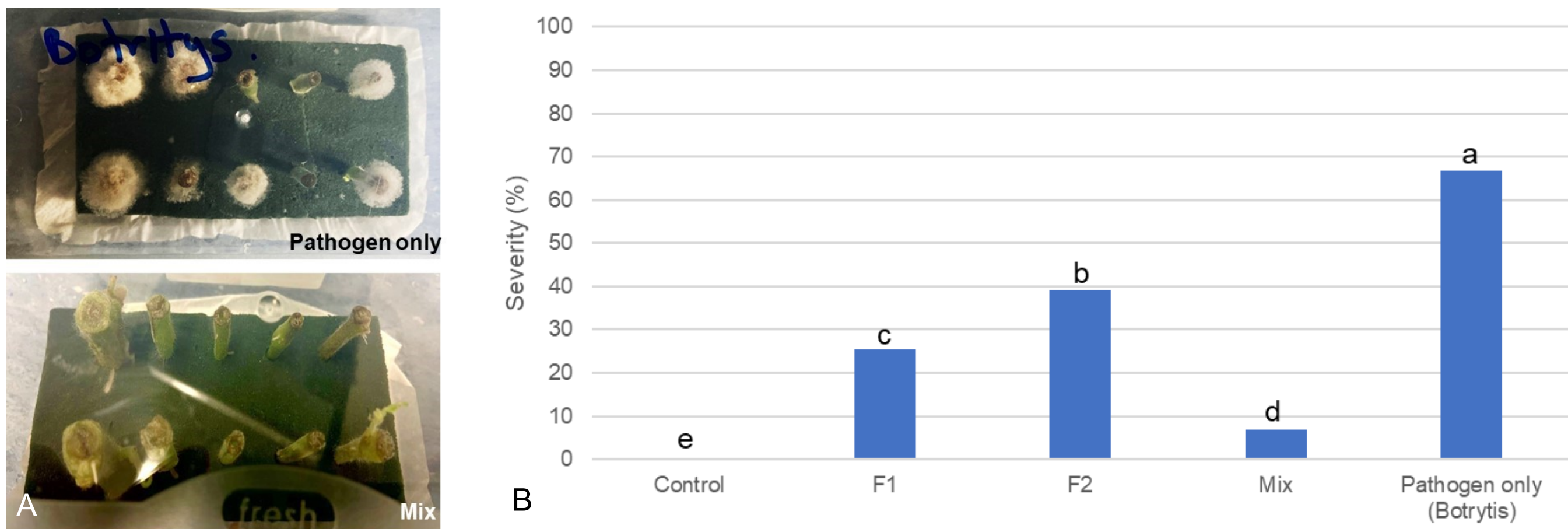


Figure 4. Tomato steams infected by *B. cinerea* (A); Effect of co-cultivated beneficial fungi (Mix) on *B. cinerea* severity on tomato steams (B)
Treatments with the same letter are not significantly different according with Tukey test (95%)

- Effect of co-culture on photostability:**

The Mix culture presented better photostability when exposed to UV-C, with no viability loss after 1 minute and 30% reduction after 5 minutes of irradiation. F2 and F1 significantly ($P<0.05$) dropped viability by 4.7% and 7.5% after 1 minute and by 37% and 56% after 5 minutes of irradiation, respectively (Figure 5).

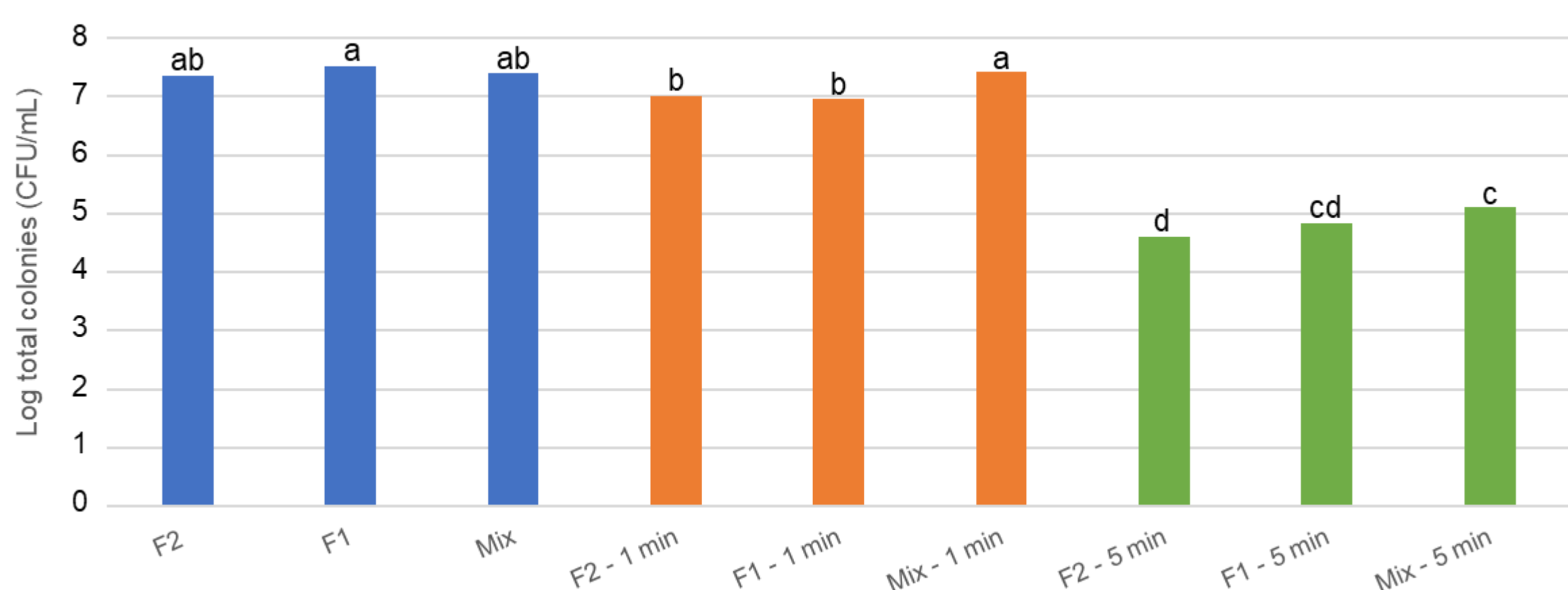


Figure 5. Fungal survival after irradiation with UV-C light
Treatments with the same letter are not significantly different according with Tukey test (95%)

Discussion

Co-culture is a proven method for uncovering metabolic pathways which are otherwise hidden in axenic culture. DNA repair pathways may have been activated in co-culture resulting in improved photostability¹. The greater suppression of the pathogen when using the two co-cultivated fungi was possibly due to the fact that they colonized the stem more quickly as they were competing for space and nutrients, which subsequently prevented pathogen entry more efficiently².

Conclusion

The co-culture of beneficial fungi is a useful strategy to enhance fungal traits and could be implemented to develop more efficient and persistent biopesticides.