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Mycoparasitic evaluation of native strains of *Trichoderma* spp. against *Verticillium dahliae* from the province of Catamarca (Argentinian Republic)

Abstract

The capacity as a biological control agent is due to multiple mechanisms, such as competition for nutrients, for space and myco-parasitism. While the fungus of the *Trichoderma* genus has several advantages as a Biological Control Agent; it takes nutrients from the fungi that it degrades and from the organic matter helping its decomposition. Therefore, the objective of this work was to determine through microcultures the mycoparasitic capacity of native strains of *Trichoderma* spp. against *Verticillum dahliae*, etiological agent of olive verticillium wilt. The action and type of parasitism of three native strains of Trichoderma against the phytopathogen *V. dahliae* was determined using the microculture technique. A trial with a completely randomized design with a 3x2x2 factorial arrangement (3 antagonists x 2 pH levels x 2 lighting levels) was performed. The capacity of each native strain of *Trichoderma* to exert different types of parasitism under the established conditions was determined. This being considered of great interest since the biocontrol of the phytopathogen is evidenced through physical contact with the native strain of *Trichoderma*. Therefore, it is of great importance to continue the antagonism studies of different strains of *Trichoderma* to determine the efficiency of the control of *V. dahliae*.

Keywords: microcultures, olive Verticillium wilt, pH, photoperiod

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Introduction

The determination of Biological Control Agents requires carrying out specific antagonism studies on the various phytopathogens.[1]

Trichoderma is the genus of a free-living, facultative aerobic, plant symbiont, avirulent fungus that belongs to the *Hypocraceae* family and has more than 30 species, all with beneficial effects for agriculture[2] and is characterized by not present a certain sexual state.[3,4] *Trichoderma* reproduces asexually with mycelium and an abundant amount of green conidia (or spores), formed from the naked cells of its fruiting body, it is fast growing and has extensive enzyme production.[5] The conidia are ovoid in shape, formed from highly branched and septate conidiophores. It also has resistance structures called chlamydospores formed on the mycelium with a thick and rough cell wall. The species most used for Biological Control are *T. atroviride; T. harmatum; T. asperellum* and *T. harzianum*.[6]

This genus can act as a biocontrol agent in an effective way, being low-cost in production, does not cause environmental damage and does not harm useful organisms that contribute to the balance of the environment.[7,8] According to studies carried out, *Trichoderma* was a fungus with efficiency to reduce phytopathogens that cause infections, such as those that cause vascular diseases or foliar diseases in plants.[5,9] These characteristics mean that the correct choice of a promising strain of *Trichoderma* in accordance with the desirable qualities that it presents, generates benefits in the modes of action and ecological aptitudes, these being specific to the species or strain to develop its full potential in a successful commercial product.[3,5] Its capacity as a biocontroller is due to the multiple mechanisms it possesses it generates competition for nutrients, space and myco-parasitism, [3,8,10-13] among others. In turn, *Trichoderma* has several advantages as a Biological Control Agent; it takes nutrients from fungi (which it degrades) and from organic matter helping its decomposition, for which the incorporation of organic matter and composting favor it.[2]

The antagonistic action mechanism of *Trichoderma* was described in the 1970s by Weindling,[14] currently indirect and direct actions are known, the latter regulate the development of phytopathogenic fungi[15-17] such as: mycoparasitism, antibiosis, competition (nutrient and space), being for Guedez et al.[18] mycoparasitism the main mechanism of action of these fungi and the best known.[10] The antagonists can present more than one way of acting; this is of great interest at the time of choosing the Biological Control Agent,[16,19] in this way the resistance of the strains.[20] So, the mechanisms depend on each strain of *Trichoderma* and the environmental conditions. Among them is Mycoparasitism, which is the direct impact of *Trichoderma* on another species of fungus. There are currently 75 species of *Hypocrea/ Trichoderma* known to have this ability. The processes described below occur sequentially and continuously:[8,15,21,22]

Chemotrophic growth: It is the direct growth towards a chemical stimulus. In the host localization stage, *Trichoderma* can detect the phytopathogen from a distance and its hyphae grow towards it.

Recognition: Recognition is carried out through lectin-carbohydrate interactions, finding carbohydrates present in the cell wall of *Trichoderma*, while lectins are present in phytopathogens. Then the process continues with the development of hyphae and appressoria. According to research, these are effective only against specific phytopathogens, so molecular recognition between Trichoderma and the host (phytopathogen) is essential for antagonism.

Adhesion and coiling: After recognition, the *Trichoderma* hyphae adhere to those of the host through hyphae and appressoria that coil around the host, the adherence of the *Trichoderma* hyphae occurs through the carbohydrate-lectin association.

Lytic activity: In this stage there is production of extracellular lytic enzymes (chitinase, cellulase, glucanase and proteases), which degrade the host cell wall and resistance structures that allow the penetration of the antagonist's hyphae, as well as facilitate

the insertion of specialized structures (hyphae, haustoria) for the absorption of nutrients from the interior of the phytopathogen. Finally, mycoparasitism ends with the loss of the cytoplasmic content of the host cell. The remaining cytoplasm is found surrounding the invading hyphae, with symptoms of disintegration, retraction of the plasma membrane and disorganization of the cytoplasm [12,15,16,18].

Some of the investigations show the action of *Trichoderma* against *Rhizoctonia solani, Alternaria alternata; Sclerotinia sclerotiorum, Fusarium spp., Botrytis cinerea, Pythium spp*. and *Ustilago maydis* where the deterioration of phytopathogens is confirmed.[8] In studies through microscopic observations, they highlight that it is not always feasible to visualize these interactions, since it depends on the *Trichoderma* isolate and the phytopathogenic agent in question.[16] Therefore, the objective of this work was to determine through microcultures the mycoparasitic capacity of native strains of *Trichoderma* spp. against *V. dahliae*, etiological agent of olive verticillium wilt.

Materials and methods

The action and type of parasitism of three native strains of *Trichoderma* against the phytopathogen *V. dahliae* was determined using the microculture technique. A trial with a completely randomized design was carried out with a 3x2x2 factorial arrangement, the first factor "antagonists" with three strains of *Trichoderma*, a second factor "pH" with two pH levels (6.5 and 4.5) and the third "photoperiod" factor with two lighting levels (8 and 16 h of light).

The microcultures were carried out following the technique of Martins Corders and de Melo.[23] Petri dishes of 15 cm in diameter were used as a humid chamber, in which sterile slides were conditioned and on them a drop of sterile ADP culture medium with the established pH was placed, on which the phytopathogen contained in a 5 mm disc was sown. of Ø of ADP in active growth, a coverslip was placed and after 48 h of incubation, the strains with antagonistic potential were planted in front: *Trichoderma asperellum* (VL1 and PaM3) and *T. hamatum* (M5A), contained in a 5 ADP disc. mm of Ø in active growth. Subsequently, it was incubated under controlled conditions with a T° of 25 ± 1°C and under the determined lighting conditions. Daily evaluations were carried out to determine the type of parasitism and the mycoparasitic characteristics they presented, for which cotton blue was added, and it was observed with an optical microscope with a 40x objective.

Results

The results obtained are presented in Table 1. In the treatment with *T. hamatum* (Vert + M5A) it was observed that in a photoperiod of 8 h light with pH 6.5 both strains presented fruiting (Figure 1), while with the same pH and at 16 h light in the combined treatment with *T. asperellum* (Vert + VL1) only fruiting was observed in the phytopathogen. Meanwhile, with a photoperiod of 8 h light and pH of 4.5, the treatment with the phytopathogen and the strain with *T. asperellum*, (Vert + PaM3), also presented fruiting. The action exerted by the M5A strain on *V. dahliae* is observed in Figure 2.

Table 1 Evaluation of the hyphal interaction between antagonistic strains of *Trichoderma asperellum* (VL1 and PaM3) and *T. hamatum (*M5A) with *V. dahliae*

Incubation Conditions		Vert + VL1	Vert + PaM3	Vert + M5A
Photoperiod	pH			
8 h light	4,5	CP- E- C- MP- H	H - CP - C – MP- E	CP - C – MP - H
	6,5	H- MP - CP - E	CP - H	C
16 h light	4,5	CP- H - C - MP	CP- H - MP - E	C - MP
	6,5	H - CP – MP- C	CP - H - MP - E	CP - C - MP

References: CP, parallel growth; C, coiling; MP, mycoparasitism; H, haustoria; E, crossing over.

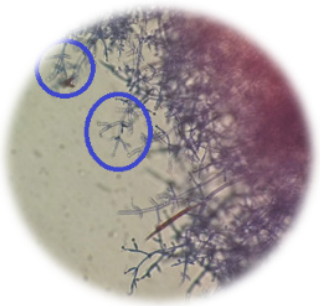

Figure 1 Whorls and conidia of *V. dahliae* under conditions of 8 h light and pH 6.5.

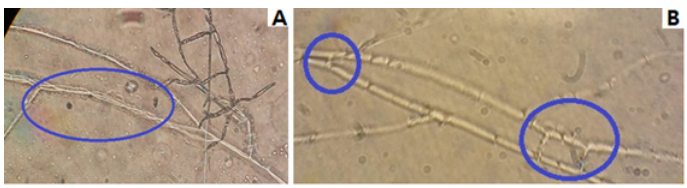

Figure 2 Strain M5A: A, coiling; B, mycoparasitism, emission of haustoria.

Discussion

The similar results obtained by Alonso Bahena[24] with *V. dahliae* were observed in the two strains of *T. asperellum* (VL1 and PaM3) under the evaluated conditions, where he also observed recognition, adhesion, parasitic symbiosis, and penetration with haustoria. Also, Rajani et al.[25] found results like those of this study. This coincides with what was stated by García Velasco et al.,[26] and according to what was stated by Zin, and Badaluddin,[27] the antagonist recognizes the phytopathogen, binds to the hyphae by appressoria and subsequently degrades the cell wall by secreting different enzymes. While other authors[26,28,29] in studies carried out only found rolling. This could be due to the lack of specificity of the *Trichoderma* strains evaluated against the phytopathogen in question, that it is not in the optimal incubation conditions to express its antagonistic potential, or another possibility that it presents a good antagonistic behavior through other properties. not expressed through these results as the metabolites, both volatile and diffusible.

Conclusion

The ability of each native strain of *Trichoderma* under study to exert different types of parasitism under the established conditions was verified. This being considered of great interest since the biocontrol of the phytopathogen is evidenced through physical contact with the native strain of *Trichoderma*. Therefore, it is of great importance to continue the antagonism studies of different strains of *Trichoderma* to determine the efficiency of the control of *V. dahliae*.

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None.

Conflicts of interest

Authors declare there are no conflicts of interest.

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