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Full Length Research Paper

Evidence of antagonistic interactions between rhizosphere and mycorrhizal fungi associated with *Dendrocalamus strictus* (Bamboo)

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The paper deals with interactions of some microfungal strains isolated from rhizosphere soils from three different sites with ectomycorrhizal fungus *Cantharellus tropicalis* mycelium grown *in vitro* on agar plates. The rhizospheric fungi were isolated from 3 different sites of bamboo forest and grown against *Cantharellus.* The cross inoculation method showed that *C. tropicalis* was highly active against some fungi, thus resulting in different types and strength of interactions. Overgrowth was the most common interaction (45%), followed by inhibition at distance (29%), intermingling (17%) and contact inhibition (13%). The competitive strength of the ectomycorrhizal fungus was high and only affected by some fast growing sterile mycelia, an unidentified fungus and *Trichoderma viride.*

Key words: *Cantharellus,* ectomycorrhiza, mycorrhizal systems, biological control, soil micro fungi.

INTRODUCTION

The microbiota of forest soils is dominated by ectomycorrhizal (ECM) and saprotrophic decomposer fungi involved respectively in supply of nutrients to trees and decomposition of woody plant litter. Saprotrophic basidiomycetes are also abundant in bamboo forests (Sharma, 2008) degrading cellulose, lignin and ligno-cellulose. Ectomycorrhizal fungal mycelia are ubiquitous in forest soils and associate with host trees to fulfill various ecological functions. Each ectomycorrhizal fungus with its special physiology can use either in-organic nutrients or utilize organic sources. In addition to increasing absorptive surface area of root systems, ECM fungi provide an increased surface area for interactions with other microorganisms, thus translocating products of photosynthesis to soil. These interactions may be inhibitory or stimulatory, some are clearly competitive, others mutualistic. An understanding of interactions between ECM and saprotrophic organisms is important given their central roles in biogeochemical cycling in ecosystems of both managed and natural forests. However, saprotrophs obtain their C from decaying organic matter while ECM fungi obtain most of their C directly from their host plants (Leake and Johnson, 2004). Antagonistic interactions between rhizosphere microorganisms and mycorrhizal fungi have an important role in functions of mycorrhizal systems (Stark and Kytöviita, 2005). Moreover, exudation and re-absorption of fluid droplets at ECM hyphal tips helps in conditioning the hyphal environment in the vicinity of tips (Sun et al., 1999).

Mycorrhizal fungi also modify the interactions of plants with other soil organisms, both pathogens (nematodes and fungi) and mutualists (nitrogen-fixing bacteria). Path-ogenic fungi, may invade roots and mycorrhizal fungi can alter host response to these pathogens. *Laccaria bicolor* prevented the spread of *Fusarium oxysporum* in Douglas-fir roots as a result of flavanoid wall infusions (Fitter and Garbaye, 1994). Wu et al. (2003) explored interactions between saprotrophic microbes and ECM fungi using a protein-tannin complex as N source by red pine (*Pinus resinosa*). Olsson (1999) studied the role of fatty acids to determine the distribution and interactions of mycorrhizal fungi in soil. Mycorrhizal fungi colonize feeder roots and thereby interact with root pathogens that parasitize them. In a natural ecosystem where uptake of phosphorus is low,

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mycorrhizal fungi protect root system from endemic pathogens such as *Fusarium* spp. Mycorrhizal fungi may reduce the incidence and severity of root diseases (Whipps, 2004). Over the last 30 years, there has been an increasing interest in potential role that ECM fungi can play in control of plant diseases. It is possible to exploit these interactions to improve mycorrhizal function (Finlay, 2004) and restrict pathogenic organisms in the form of biological control.

There have been a few laboratory studies of interactions between pure cultures of representatives of both ECM and saprotrophs fungi in axenic microcosm system**.** In the same way no remarkable studies of competitive interactions between mycorrhizal *Cantharellus tropicalis* Rahi, Rajak and Pandey and saprotrophic fungi in soils have been done. The objectives of the present study were to examine whether interactions occur between species of different fungal group from bamboo forest. Interactions between ectomycorrhizal fungi and rhizospheric soil microfungi were studied *in vitro*, providing us an insight into the ecology of ectomycorrhizal fungi associated with *Dendrocalamus.*

MATERIALS AND METHODS

Rhizosphere soil samples of *D. strictus* were collected from three sites of bamboo forests in the districts of Balaghat (site 1), Lamta (site 2), and Nainpur (site 3), district Balaghat, Madhya Pradesh, India. With the help of a trowel the samples were collected at a depth of 5 – 10 cm along with root bits into sterile polythene bags. The composite soil samples were immediately brought to the laboratory and stored in refrigerator at 4±2°C for further analysis in order to determine the soil type and nutritional status.

The cross inoculation methods used by Baar and Stanton (2000) and Vaidya (2005) was followed with modifications. MMN (modified Melin Norkrans) medium with agar (15 gl^{-1}) was used for growth of all fungi. Many ECM grow well on MMN media. Also, soil micro fungi grow well on MMN medium. For inoculation, mycelial plugs of 9 mm diam. were cut from edge of ECM mycelia and transferred to MMN agar. Pair-wise combinations were made by plating mycelial plugs of ECM and soil micro fungi on opposite corners of plate, about 70 – 80 mm away from each other. Each pair-wise combination was replicated three times. Agar plates were incubated at 28±2°C. Radial growth towards other mycelium was determined by measuring colony radius. The experiment was terminated when radial growth of ECM fungi reached other mycelium.

RESULTS

The cross inoculation method has shown that *C. tropicalis* was highly active against some rhizosphere soil microorganism. Pair-wise combinations of ECM *C. tropicalis* and soil micro fungi from sites (site 1 - Figures 1, 2; site 2 - Figures 3 and 4; site 3 - Figures 5 and 6) differed between species. This resulted not only in different types of interactions between fungi, but also in differences in strength of interactions. Overgrowth was the most common interaction (45%), followed by inhibition at a distance (29%) intermingling (17%) and contact inhibition (13%). Details of observation are given in Table 1.

Overgrowth was observed when *C. tropicalis* interacted with *A. flavus Link ex Fr.*. Inhibition at a distance was observed for combinations between *C. tropicalis* and either *Aspergillus niger* Link ex Fr. or *Emericella* sp. Isolated from site 1 (Figures 1c and 2a); *Aspergillus* sp. 2 isolated from site 2, however, the mycelia of *Aspergillus* sp. 2 were larger and less suppressed by *C. tropicalis* (Figures 3a); and similar results were observed for *Aspergillus* sp. 1 isolated from site 3 (Figure 5a).

Sterile mycelium from site 3 overgrew *C. tropicalis* (Figures 6b and 6c). Both species of *Trichoderma* spp. (site 1 and 2) restricted the growth of chanterelle. However, when chanterelle was grown in combination with *A. niger* Van Tiegh*,* a clear zone with no hyphae of either fungi was formed (Figures 1a, 5c and 5d).

DISCUSSION

The inhibition of soil micro fungi, mostly at a distance by *C. tropicalis*, suggests that this fungus prevented invasion by potential competitors. This type of defense mechanism has been reported for other ectomycorrhizal fungi (Baar and Stanton, 2000). Herein, the inhibition of soil micro fungi by *C. tropicalis* might be caused by production of secondary metabolites. However, antibiotics produced by ECM species (that is, *Amanita, Boletus* and *Cenococcum* spp.) have been reported in earlier studies (Santoro and Casida, 1962).

The overgrowth of *C. tropicalis* by relatively fast-growing sterile mycelial fungus, unidentified fungus and *Trichoderma viride* Pers. ex Fr. was remarkable. The results of an earlier study by Shaw et al. (1995) showed growth suppression of *Rhizopogon roseolus* by several saprotrophic basidiomycetes. Furthermore, the growth of *Suillus granulatus* (L.:Fr.) Rouss*,* was inhibited by rhizoplane fungi of *Pinus halepensis* (Girlanda et al., 1995). Baar and Stanton (2000) have attributed low investment of N in mycelial biomass to reduced competition for some ECM fungi. Hardly any sporocarps of saprotrophic basidiomycetes occur in bamboo forest but species of *Ramaria, Clavaria* and *Clitocybe* have been found growing near the bamboo plants, but could not be isolated. They can be studied for their competitiveness with chanterelle. In previous studies, *Clitocybe marginella* Harmaja inhibited the growth of *Cenococcum geophilum* and *L. bicolor* (Baar and Stanton, 2000). In a similar microcosm experiment in which mycelium of *Suillus bovinus* (L.:Fr.) Rouss mycorrhizal with *Pinus sylvestris* were grown alone and in interaction with *Phanerochaete velutina* (Leake et al*.,* 2001), the effect of mycorrhiza on growth of saprotroph was limited.

In the present study, the ectomycorrhizal fungi suppressed the soil micro fungi in maximum number of the pair wise comparisons indicating that *Cantharellus* mycelia has higher competitiveness than soil micro fungi. This may be attributed to several alkaloids, terpenes,

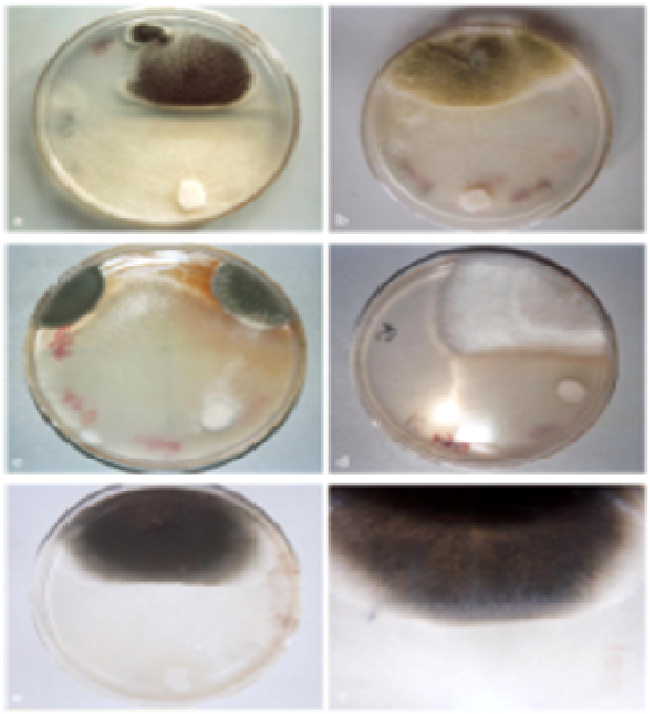

Figure 1. Dual culture interaction between *C. tropicalis* (*Ct*) and soil microfungi (site 1). a. *Ct-Aspergillus niger*, b. *Ct-A. terreus*, c. *Ct-A. flavus*, d. *Ct-Fusarium* sp.1, e. *Ct-Curvularia* sp., f. enlarged zone of *Ct-Alternaria* sp. interaction.

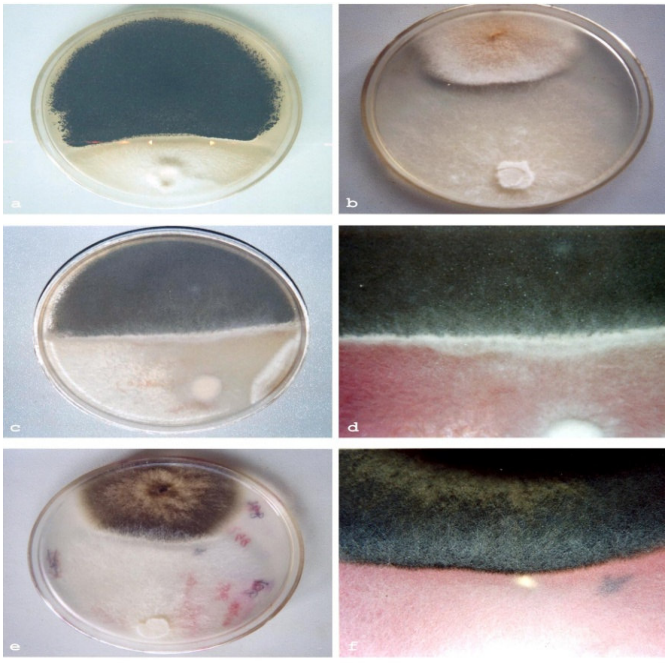

Figure 3. Dual culture interaction between *C. tropicalis* (*Ct*) and soil microfungi (site 2- Lamta). a. Ct-*A.niger*, b. *Fusarium* sp.1, c. Ct-*Alternaria* sp., d. enlarged zone of Ct-*Alternaria* sp. interaction, e. *Ct-Curvularia, f.* enlarged zone of *Ct-Curvularia* sp. interaction.

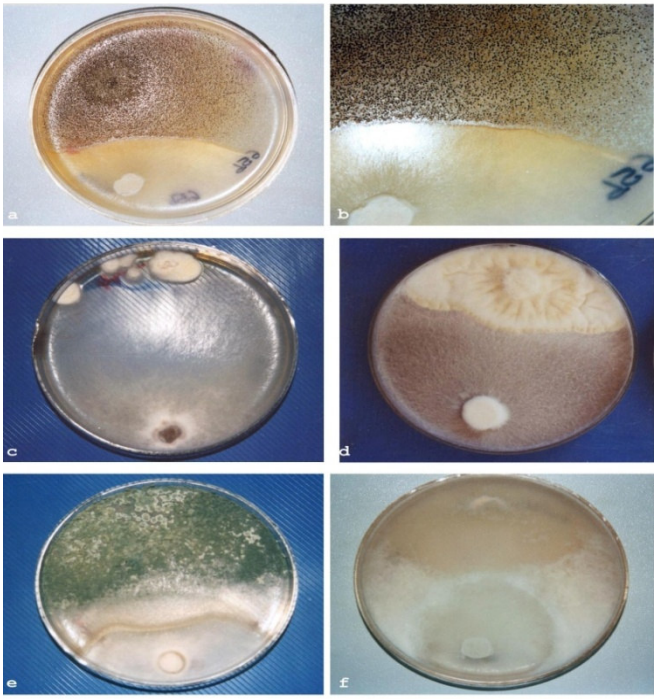

Figure 2. Dual culture interaction between *C. tropicalis* (*Ct*) and soil microfungi (site 1- Balaghat). a. *Ct-Emericella* sp., b. enlarged zone of *Ct-Emericella*interaction, c. *Ct-Penicillium* sp.1, d. *Ct-Fusarium* sp.2, e. *Ct-Trichoderma viride*, f. *Ct*-Unidentified fungus.

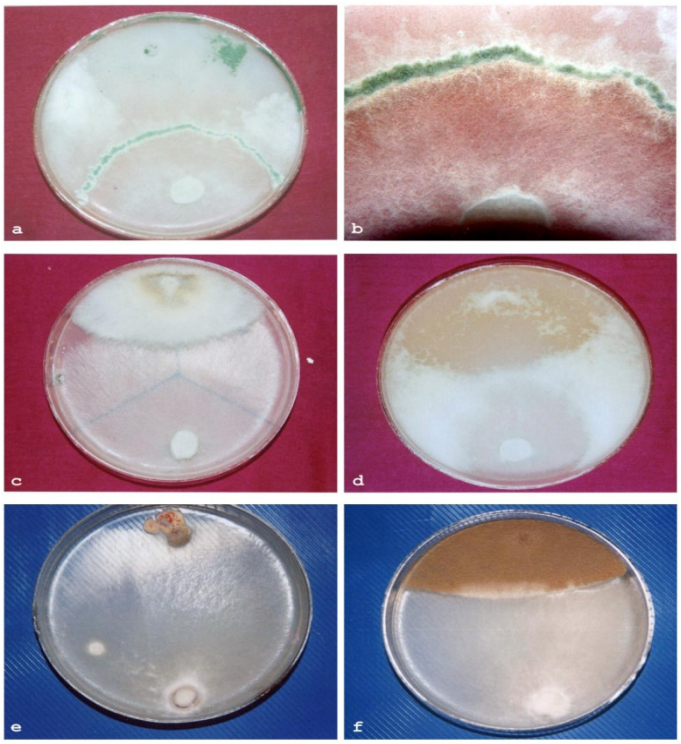

Figure 4. Dual culture interaction between *C. tropicalis* (*Ct*) and soil microfungi (site 2- Lamta). a. *Ct-Trichoderma* sp., b. enlarged zone of *Ct-Trichoderma* sp. interaction, c. *Ct-Fusarium* sp.1, d. *Ct*-Unidentified fungus, e. *Ct-Penicillium* sp.3, f. *Ct-Aspergillus* sp.2.

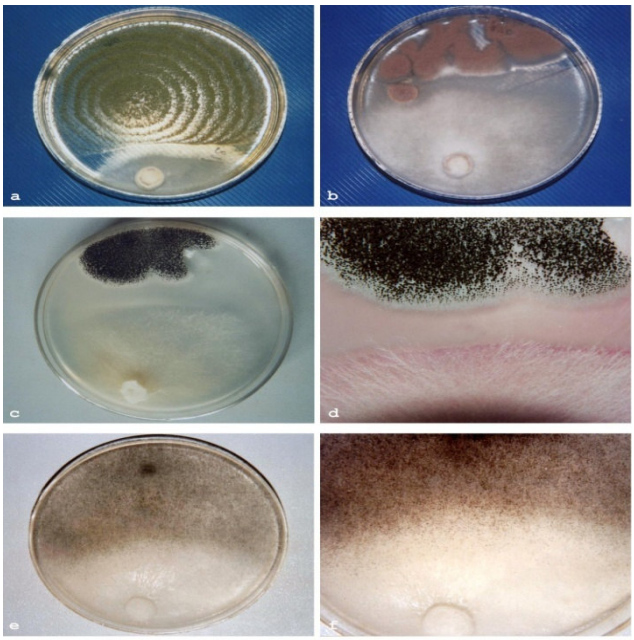

Figure 5. Dual culture interaction between *C. tropicalis* (*Ct*) and soil microfungi (site 3- Nainpur). a. *Ct-Aspergillus* sp.1, b. *Penicillium* sp.1, c. *Ct-A. niger*, d. enlarged zone of interaction, e. *Ct-Mucor* sp., f. enlarged zone of Ct-*A. flavus* interaction.

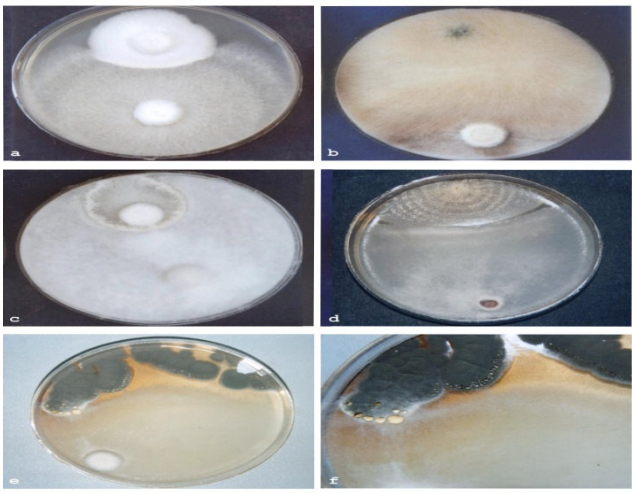

Figure 6. Dual culture interaction between *C. tropicalis* (*Ct*) and soil microfungi (site 3 - Nainpur). a. *Ct-Fusarium* sp.1, b. Sterile mycelium, c. *Ct*-Unidentified fungus, d. *Ct-Emericella* sp., e. *Ct-A. flavus*, f. Enlarged zone of *Ct-A. flavus* interaction.

polysaccharides produced by *Cantharellus* mycelia. Different strategies were observed for soil micro fungi such as inhibitor at a distance (29%), contact inhibition (13%), intermingling (17%) and overgrowth (45%). *Penicillium* sp., a known mycotoxins producer was hardly combative against the ECM fungus (Table 1, Figures 2c, 4e and 5b). Low competitiveness of some of the soil micro fungi *viz., Curvularia, Alternaria, Mucor* and *Fusarium* may suggest that these species occupy different niches or are weak organisms when competing with *Cantharellus*. Moreover there are seldom reports of any root disease in *Dendrocalamus.*

ECM fungi have been shown to have inhibitory effects on root pathogenic fungi but their interactions with saprophytic fungi have received surprisingly little attention (Johansson et al, 2004). There has been reports of strong inhibition of root pathogens like *Phytophthora cinnamomi* Rands, *Pythium debaryanum* Hesseltine and *P. sylvaticum in vitro* by ectomycorrhizal fungi. Stark and Kytöviita (2005) provided evidence of antagonistic interactions between rhizosphere microorganisms and mycorrhizal fungi associated with birch (*Betula pubescens* Ehrh.). Isolates of *Laccaria* sp. protected young seedlings of *Picea abies* (L.) Karst. and *Pseudostuga menziesii* from *F. oxysporum* (Sampangiramaiah and Perrin, 1990). Natarajan and Govindaswamy (1990) have tested *Amanita muscaria, Laccaria laccata, L. fraterna,* and *Suillus brevipes* against six root pathogens *viz., Armillaria mellea* (Vahl in Fl. Dan. ex Fr.), *Cylindrocladium parvum* Anderson*, C. scoparium* Morg.*, F. oxysporum* Schlecht.*, F. solani* (Mart.) App. and Wollenw and *Rhizoctonia solani* Kuehn *S. brevipes* inhibited all root pathogens tested. In another study, *Tricholoma* sp., *Paxillus involutus* and *Hebeloma cylindrosporum* inhibited growth of *Cylindrocladium floridanum* in Petri dishes, while *L. bicolor* was inhibited and completely covered by *C. floridanum* (Morin et al., 1999). Growth (in paired culture) and colony forming units (in the rhizosphere of *Pinus banksiana* Lamb. seedlings) of *F. oxysporum* was reduced significantly by *L. laccata*. When grown in co-culture, Werner and Zadworny (2003) observed suppression of *Mucor hiemalis* by *L. laccata.* They also studied interactions between the *L. laccata* and soil fungus *Trichoderma virens* in co-culture and in the rhizosphere of *P. sylvestris* seedlings growing *in vitro,* where growth of *T. virens* was inhibited in co-culture (Werner et al., 2002). Antifungal and antibacterial action of ECM fungi *Pisolithus* and *Scleroderma in vitro* have been *tested* against 8 fungi and 6 bacteria and showed higher activity against all fungi except three *Aspergillus* spp. (Vaidya et al., 2005).

While our knowledge is currently limited, it seems that interactions have profound effects on mycorhizosphere processes. The ability to redistribute nutrients between compartments of forest floor is a fundamental activity of many saprotrophic and mycorrhizal fungi. More extensive research is warranted to enhance our knowledge on interactions within fungal community and exploring potential for manipulating ectomycorrhizosphere environment for biotechnological purposes (Bruns and Bidartondo, 2002; Cairney and Meharg, 2002). The intensity of interactions between different soil fungi and ECM fungus *C. tropicalis* highlights the potential importance of interactions on functioning of these microorganisms in forest ecosystems.

Table 1. Results of the fungal interactions and estimated average size (% of Petri dish covered) of the mycelia of *C. tropicalis* (*Ct*) with soil micro fungi at the harvest time of the three sites studied (site 1 - Balaghat; site 2 - Lamta; site 3 - Nainpur).

S/No.	Interactions	Site 1†*		Site 2†*		Site 3†*	
		Fungal interaction	% PD covered	Fungal interaction	% PD covered	Fungal interaction	% PD covered
1	*Ct-A. alternata*	-	-	O (s)	47 - 53	-	-
2	*Ct-A. flavus*	O (e)	81 - 19	-	-	O (e)	68 - 32
3	*Ct-A. niger*	HD (e)	52 - 48	HD (s)	32 - 68	HD (e)	58 - 42
4	*Ct-Aspergillus* sp.1	CH (e)	58 - 42	-	-	CH (s)	29 - 71
5	*Ct-Aspergillus* sp.2	-	-	CH (e)	62 - 38	-	-
6	*Ct-Curvularia* sp.	O (e)	55 - 45	O (e)	56 - 44	-	-
7	*Ct-Emericella* sp.	HD (s)	39 - 61	-	-	HD (s)	67 - 33
8	*Ct-Fusarium* sp.1	O (e)	56 - 44	O (e)	66 - 34	O (e)	61 - 39
9	*Ct-Fusarium* sp.2	O (e)	51 - 49	O (e)	62 - 38	-	-
10	*Ct-Mucor* sp.	-	-	-	-	M (s)	44 - 56
11	*Ct-Penicillium* sp.1	CH (e)	83 - 17	-	-	-	-
12	*Ct-Penicillium* sp.2	-	-	-	-	HD (e)	60 - 40
13	*Ct-Penicillium* sp.3	-	-	HD (e)	80 - 20	-	-
14	*Ct-T. viride*	O (s)	28 - 72	-	-	-	-
15	*Ct-Trichoderma* sp.	-	-	O (s)	46 - 54	-	-
16	*Ct*-Sterile mycelium	-	-	-	-	M (s)	22 - 78
17	*Ct*-Unidentified fungus	M (e)	49 - 51	M (e)	48 - 52	M (e)	61 - 39

* Interactions distinguished were: contact inhibition (CH), inhibition at distance (HD), intermingling (M), and overgrowth (O). † Letters in brackets indicate which fungus exerted a specific interaction effect upon its opponent: ECM fungus (e), soil micro fungus (s). Dash (-) indicates that particular soil micro fungus was not isolated from that site.

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