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# *In vitro* studies on biological control of *Phytophthora meadii* using *Trichoderma* spp.

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## ABSTRACT

An attempt was made to study the effect of antagonistic fungi, *Trichoderma viride, T. koningi* and *T. harzianum,* against *Phytophthora meadii* which causes abnormal leaf fall disease in rubber *(Hevea brasiliensis). In vitro* screening of these antagonists showed that all inhibited growth of the pathogen. The antagonists were also found to penetrate the oospores of the fungus and cause their lysis.

## Introduction

Abnormal leaf fall, caused by *Phytophthora meadii,* is a major disease in rubber which accounts for 38 to 56% of crop loss[1]. However, a recent systematic study indicated a crop loss of only 9 to 16% when annual protection measures are not undertaken for one disease season[2].

The modern approach in disease management is the use of biocontrol agents which are cheap and free from environmental pollution. Smith *et al*[3] demonstrated the efficacy of biological control of *Phytophthora* related root and crown rot of apples by *Trichoderma* spp. Isolates of *Trichoderma* spp. are known to cause lysis of oospores and cysts of *Phytophthora cactorum* in dual culture on Potato Dextrose Agar (PDA)[4]. The present investigation was therefore carried out to study the efficacy of *Trichoderma* spp. in the control of *P. meadii.*

## Materials and methods

### *Inhibition of the growth of P. meadii by Trichoderma spp.*

The antagonistic effect of *Trichoderma viride, T. koningi, T. harzianum, T. kiningi* (native isolate), *T. hamatum,* and *T. harzianum* (native isolate) were studied by dual culture techniques. The test organisms were placed on one end of a PDA medium. A 6mm diameter mycelial disc of *P. meadii* was placed on the other end of the PDA in the Petri dish in such a way that they were 7cms apart and then incubated at 28 ± 2°C. The mycelial growth of *P. meadii* and the antagonist was recorded in each case[5].

### *Effect of antagonists on oospores of P. meadii*

A set of Petri plates containing PDA medium were inoculated with the antagonistic cultures and a 5mm diameter well was made with a cork borer in the medium at the centre of each plate. A portion of mycelia of the paired culture having oospores of *P. meadii* was transferred to the well and the antagonists were allowed to grow. The oospores were examined under the microscope after the antagonists had completely overgrown. A similar study was carried out using sterile soil in place of the PDA medium. Sterile soil in plastic cups was inoculated with cultures of *Trichoderma* spp., moistened and then allowed to stabilize for 3 days. Small

portions of mycelia of the paired culture of *P. meadii* having oospores were kept in tissue paper packets which were buried in the *Trichoderma* spp. culture-inoculated soil. The soil was kept moist for one week. The oospores of the pathogen was then retrieved and examined for penetration of the antagonist.

**Results and discussion**

***Inhibition of the growth of P. meadii by Trichoderma spp.***

The results of the study on the antagonistic effect of the six isolates of *Trichoderma* against *P. meadii* are presented in Table 1. All the antagonists tested were found to inhibit the growth of *P. meadii* in various degrees. However, the maximum antagonistic effect was shown by *T. viride* followed by *T. koningi* and the others. The hyphae of *Trichoderma* spp. overgrew the pathogen and continued to grow closely with the host hyphae. Similar overgrowth of antagonists was reported earlier by Mukherjee *et al*[6]. The present investigation showed that the hyphae of *P. meadii* was tightly held by the coiling slender hyphae of the antagonists. The antagonistic hyphae penetrated the host hyphae at several points and grew inside the mycelia of the host. Similar mycoparasitic activity of *T. harzianum* with *Fusarium oxysporum* and with *R. solani* has been reported previously[7]. Besides coiling and overgrowth, the antagonistic microorganisms also caused lysis of *P. meadii* on the PDA medium. The antagonistic hyphae cause lysis of the hyphae of *Pythium vexan* and *R. solani* due to secretion of some cell wall dissolving enzymes by antagonists [8,9,10].

**Table 1** ***Effect of Trichoderma spp. on P. meadii causing abnormal leaf fall disease.***

| Treatments | Mean mycelial growth of pathogen in mms | Mean mycelial growth of antagonists in mms |
|---|---|---|
| 1. *Trichoderma viride* | 1.1 | 8.3 |
| 2. *Trichoderma koningi* | 1.3 | 8.1 |
| 3. *Trichoderma harzianum* | 1.4 | 8.1 |
| 4. *Trichoderma koningi* (native isolate) | 1.5 | 7.9 |
| 5. *Trichoderma hamatum* | 1.5 | 7.8 |
| 6. *Trichoderma harzianum* (native isolate) | 1.9 | 7.3 |
| 7. Control | 6.7 | - |
| C.D. (P = 0.05) | 0.4 | 0.04 |

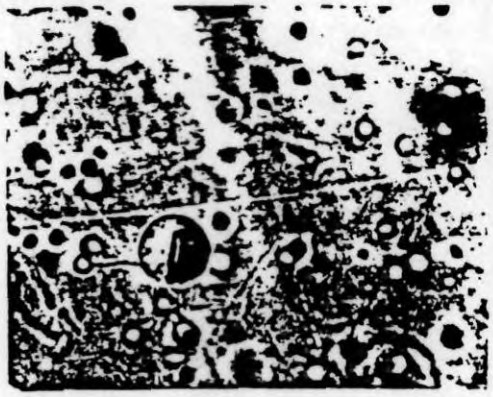
***Oospore penetration and oospore lysis***

The antagonistic hyphae were found to penetrate the oospores of *P. meadii* and cause lysis of oospores; 56 out of 86 oospores were lysed whereas only 3 out of 153 oospores observed were lysed in the control (Table 2, Plate 1). Phillips[11] found that *Gliocladium virens* parasitised and decayed the sclerotia of *M. phaseoline* on media and reduced the survival of sclerotia. Elad *et al*[12] observed that four isolates of *T. harzianum* inhibited linear growth and microscleratia production of *M. phaseolina in vitro*. The antagonist proliferates in dual liquid cultures with the pathogen and significantly reduced the number of its viable propagules.

**Table 2** *Effect of Trichoderma spp. on lysis of oospores of Phytophthora meadii.*

| Treatment | Number observed | Number lysed | % lysed |
|---|---|---|---|
| *P. meadii + Trichoderma spp.* | 86 | 56 | 65.11 |
| *P. meadii* | 153 | 3 | 1.96 |

**Plate 1** *Lysis of an oospore of Phytophthora meadii by Trichoderma spp.*



An isolate of *T. harzianum* (IMI No.238493) directly attacked and lysed the mycelium and sclerotia of *Sclerotium rolfsii* when both fungi were grown in dual culture. The type of interactions between the antagonist and pathogen were hyphal coiling, entry through haustoria-like structures and direct entry into the hyphae and sclerotia of *S. rolfsi*[13]. Leader *et al*[4] reported the effect of ten *Trichoderma* isolates on zoospores and cysts of *P. cactorum* in dual culture on PDA. Three *Trichoderma* isolates were active against zoospore and oospore lysis due to penetration by *Trichoderma* spp. This is in accordance with the hyphal lysis, oospore lysis and oospore penetration observed in the present study.

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