Jl Rubb Res Inst. Sri Lanka (1991 71, 42-47)

SOME EFFECTS OF ISOLATES OF *TRICHODERMA* ON *RIGIDIPORUS LIGNOSUS*

By

R. L. C. Wijesundera[1], S. Jeganathan
Dept. of Botany, University of Colombo.
Colombo 3, Sri Lanka.

and

N. I. S. Liyanage*
Dept. of Plant Pathology, Rubber Research Institute,
Agalawatta, Sri Lanka.

ABSTRACT

Seven forms of *Trichoderma* were isolated from soil obtained from rubber plantations. All the isolates secreted compounds inhibitory to the growth of *Rigidiporus lignosus* the causal agent of the white root disease of rubber. Further, the *Trichoderma* isolates interacted with the hyphae of *R. lignosus*, the interactions resulting in harmful effects on *R. lignosus* hyphae. Of the isolates examined *T. Koningii* isolate IV and *T. harzianum* appear to be very promising as potential biocontrol agents.

INTRODUCTION

Rigidiporus lignosus cause the white root disease of rubber *(Hevea brasiliensis)*. It is the most destructive disease affecting the rubber tree and is of considerable economic importance. The present method available to control the white root disease is rather cumbersome since, it mainly consists of removal of infected roots and root debris. Hence, there is a need to develop alternative methods to control the white root disease (Liyanage, *et al.*, 1977).

The soil inhabiting fungus *Trichoderma* have proved successful in controlling a number of plant pathogenic fungi including root infecting forms. *Trichoderma* are known to produce chemicals which inhibit growth of other micro-organisms and in addition *Trichoderma* hyphae interacts with hyphae of others causing harmful effects (Papavizas, 1985).

---

*Presently, University of South Pacific, W. Samoa.
[1] Address for all correspondance.

In this study the effect of *Trichoderma* spp. present in rubber soils, on *Rigidiporus lignosus* were investigated with a view to developing any antagonistic effects to control the white root disease.

MATERIALS AND METHODS

Organisms:

Rigidiporus lignosus (isolate T1) was obtained from the Plant Pathology Department, Rubber Research Institute, Agalawatta. The fungus was maintained on potato dextrose agar (PDA) at 30°C.

Trichoderma was isolated from soil samples collected from the Dartonfield rubber plantation, Agalawatta, using the soil dilution cup method (Warcup, 1950) on PDA. The isolates were identified using the morphological and reproductive characters (Rifai, 1969) and the identifications were confirmed by the CMI International Mycological Institute (IMI), London. The isolates are listed in Table 1. All the isolates were maintained on PDA at 30°C.

Study of inhibitory effects:

To study the inhibition of *R. lignosus* by the *Trichoderma* isolates the following methods were used (Egorov, 1985).

(a) Agar block method:

Four 1 cm^2 agar squares obtained from the periphery of a 5–day old *Trichoderma* isolate growing on PDA at 30°C, were placed 3.5 cm from the centre, equidistant from each other, on a PDA plate. Immediately afterwards 1 cm^2 agar square from the periphery of a 7–day old culture of *R. lignosus* on PDA at 30°C was placed at the centre of the plate. The inoculated plates were incubated at 30°C.

(b) Filter paper disc method:

A known volume of the test solution was absorbed into a sterile filter paper disc (0.5 cm diameter), the discs was air dried and a single disc were placed at the centre of a PDA plate freshly inoculated with four 1 cm^2 agar squares from the periphery of a 7–day old culture of *R. lignosus* growing on PDA at 30°C. The *R. lignosus* agar squares were placed at the periphery, 3.5 cm from the centre, equidistant from each other. The inoculated plates were incubated at 30°C.

Study of hyphal interactions:

A slide culture technique was used to detect any hyphal interactions between *R. lignosus* and *Trichoderma* isolates. A clean glass slide was placed on a Z-shaped glass rod in a 9 cm diameter Petri dish and was sterilized. Afterwards, a small amount of sterile molten PDA was poured on to the glass slide and a thin agar film was made. One end of the slide was kept free of the medium to facilitate easy handling The inocula of *Trichoderma* and *Rigidiporus* (each inoculum, a 1 cm^2 agar square obtained from the periphery of 5-day old cultures growing PDA at 30°C) were placed on the slide about 1 cm apart. About 5 ml sterile distilled water was added to the petri dish and the plates were incubated at 30°C for 5 days. Observations were made at x400.

Extraction of inhibitory compounds:

Hundred ml of potato dextrose liquid medium dispensed in 500 ml Erlenmeyer flasks were used to grow the *Trichoderma* isolates to extract any inhibitory compounds secreted. Each flask with the medium was inoculated with two 1 cm^2 agar squares obtained from the periphery of a 5 – day old culture of *Trichoderma* growing on PDA at 30°C. The inoculated media were incubated at 30°C without shaking. The cultures were harvested 10 days after inoculation by filtration through Whatman no. 1 filter paper.

Each culture filtrate was extracted 3 times, each with equal volumes of ethyl acetate. The extracts were combined and dried in a rotary evaporator at 30°C. The residue was dissolved in 2 ml of ethyl acetate and inhibitory activity examined by the filter paper disc method.

Thin layer chromatography:

Ascending thin layer chromatography, using the ethyl acetate extract of the culture filtrates on 20x20x0.1 cm^3 silica gel plates (Sigma) were developed in MeOH:EtAc 8:92 (v/v) for 5-6 h in tanks equilibrated with the solvent system. Afterwards, the plates were air dried at 30°C for 24 h and the *Cladosporium* bio-assay (Smith, 1984) was carried out.

In a separate experiment the areas corresponding to the inhibitory zones observed in the *Cladosporium* bio-assay were eluted, 3 times each with 5 ml of ethyl acetate, evaporated to dryness at 30°C in a rotary evaporator and re-dissolved in 2 ml of ethyl acetate. The inhibitory activity of the re-dissolved extract was examined using the filter paper disc method.

RESULTS

When the *Trichoderma* isolates were inoculated with *R. lignosus* on PDA plates, all isolates arrested the growth of *R. lignosus*. Clear inhibitory zones were observed. With *T. koningii* isolate IV and *T. harzianum* the inhibitory zones were persistent, whereas with the other isolates the zones did not persist for more than 2–3 days.

Microscopic examination of the slide cultures clearly showed that all *Trichoderma* isolates formed coils around the hyphae of *R. lignosus*. The frequency of coil formation differed among the isolates. The *T. koningii* isolates had the highest frequency, 60 ± 5 coils per microscopic field. In all other isolates only about 20–30 coils per microscopic field were observed (the figures are the average of 10 samples). In addition to coil formation isolates caused deformations on the surface of *R. lignosus* hyphae. The deformations were most prominent near the points of coil formation.

The ethyl acetate extracts of all *Trichoderma* isolates, when examined using the filter paper disc method, inhibited *R. lignosus*. When the ethyl acetate extract was examined by the TLC-*Cladosporium* bioassay, the inhibitory zones detected were as follows; *T. koningii* isolate I.(R_f 0.68), *T. koningii* isolate II (R_f 0.79), *T. koningii* isolate III (R_f 0.81), *T. koningii* isolate IV (R_f 0.39 & 0.69), *T. harzianum* (R_f 0.40 & 0.58), *T. hamatum* (R_f 0.78) and *T. viride* (R_f 0.59). All the inhibitory compounds, when examined, after elution and concentration by the filter paper disc method, inhibted the growth of *R. lignosus*. The highest inhibition was observed in *T. koningii* isolate IV and *T. harzianum*, when the compounds with R_f 0.39 and 0.40 were used.

DISCUSSION

If is evident from the results of this investigation, that the isolates of *Trichoderma* obtained from the rubber soils has antagonistic activity against the white root fungus *R. lignosus.* The antagonistic effects are due to the secretion of compounds which inhibited growth and harmful hyphal interactions such as coil formation. *Trichoderma* is known to produce many extracellular cell wall degrading enzymes such as glucanases and chitinases (Papavizas, 1985). Hence, the wall deformations associated with coil formation is likely to be due to the activity of such enzymes. Of the isolates examined *T. koningii* isolate IV and *T. harzianum* appear to be the most promising. *T. koningii* isolate IV had the highest frequency of coil formation and it together with *T. harzianum* showed the highest inhibitory activity.

The results therefore suggest, that the *Trichoderma* isolates either directly or their products or a combination of both have the potential to be effective agents for biocontrol of the white root disease of rubber.

REFERENCES

Egorov, N. S. (1985). Antibiotics — A Scientific Approach. Mir Publishers, Moscow 456 pp.

Liyanage G. W., Liyanage, A. de S., Peiris, O. S. & Halangoda, L. (1977). Studies of the variability and pathogenecity of *Rigidiporus lignosus. Jl. Rubb. Res. Inst. Sri Lanka,* 54:59-68.

Smith, D. A. (1984). Toxicity of phytoalexins. In *Phytoalexins,* Editors, J. A. Bailey & J. W. Mansfield, pp 218-252, Blackie, London.

Papavizas, G. C. (1985). *Trichoderma & Gliocladium:* Biology, ecology and potential for biological control. *Ann. Rev. Phytopathol,* 23:23-54.

Rifai, M. A. (1969). A revision of the genus *Trichoderma. Comm.. Mycol. Inst. Mycol. Pap.* 116. 56 pp.

Warcup, J. H. (1950). The soil plate method for the isolation of fungi from the soil. *Nature,* 116: 117-120.

Table 1. List of Trichoderma *isolated from soil with their CMI-IMI herbarium nos.*

Fungus	***IMI herbarium no.***
T. koningii Isolate I	.. 328683
T. koningii Isolate II	.. 328684
T. koningii Isolate III	.. 328685
T. koningii Isolate IV	.. 328687
T. harzianum	.. 328686
T. viridae	.. 328689
T. hamatum	.. 328690