

Soil-Borne Plant Pathogens

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Chapter 51

BIOLOGICAL CONTROL OF SOIL-BORNE PLANT PATHOGENS BY *TRICHODERMA HARZIANUM*

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Abstract

Trichoderma harzianum antagonistic to *Sclerotium rolfsii* and *Rhizoctonia solani* was isolated from soil. No anti-biotic activity of *T. harzianum* towards the pathogens could be detected. When grown on the pathogen's cell walls, the fungus produced extracellular β -(1,3)-glucanase and chitinase. When applied in the form of wheat bran culture to soil infested with *Rh. solani* or *S. rolfsii* in the greenhouse, *T. harzianum* effectively controlled damping-off disease of bean, peanuts and eggplants caused by these soil-borne plant pathogens. Field experiments were carried out and a significant reduction in disease incidence was obtained. Application of PCNB at subinhibitory doses improved control of disease when applied together with the *Trichoderma* preparation.

Introduction

In recent years the increasing use of potentially hazardous fungicides in agriculture has been the subject of growing concern of both environmentalists and public health authorities. The possibility of controlling plant-pathogenic fungi by antagonistic micro-organisms added either as a substitute or as an additive to fungicides has been the subject of extensive research. Among the many potentially antagonistic soil inhabitants, members of the genus *Trichoderma* have gained considerable success (Dennis and Webster, 1971).

Recently, Hadar *et al.* (1978) found an isolate of *T. harzianum* which directly attacked the mycelium of *Rhizoctonia solani*. When applied to soil artificially infested with *Rh. solani*, a wheat-bran culture of the antagonist effectively controlled damping-off of bean, tomato and eggplant seedlings in the greenhouse. This isolate, however, was not effective against *Sclerotium rolfsii*, neither

in culture nor in the greenhouse.

In the present study the isolation from soil of another strain of *T. harzianum* capable of decreasing incidence of various plant diseases caused by both *Rh. solani* and *S. rolfsii* in naturally infested soil in the greenhouse and the field, is reported.

Material and methods

Fungi isolated from soil were tested for their antagonistic ability towards soil-borne pathogens as described by Dennis and Webster (1971), using the solidified synthetic medium (SM) of Okon *et al.* (1973). The isolate used in this work was identified as *Trichoderma harzianum* Rifai according to Rifai (1969).

Greenhouse experiments

A loamy sand soil (Hadar *et al.*, 1978), artificially infested with either *Rh. solani* or *S. rolfsii*, was used. *Rh. solani* was grown as a thin mycelial mat on yeast extract-dextrose broth (Henis and Ben-Yephet, 1970) for 8 days at 27°C. Then the mycelial mat was washed with sterile water, blotted between two sheets of filter paper, taken in 100 ml water and homogenized in a Waring Blendor for 60 sec. It was mixed with the soil at a final concentration of 250 mg wet weight/kg soil. *S. rolfsii* was grown on SM agar for 10 days. Sclerotia were harvested and mixed with the soil at a final concentration of 100 mg (fresh weight)/kg soil (dry weight).

The antagonistic isolate of *T. harzianum* was grown on a wheat-bran tap water (1:2) mixture which was autoclaved for 1 h at 121°C twice in two successive days. Erlenmeyer flasks (250 ml) containing this medium were inoculated with *T. harzianum* and incubated in the dark for 8 days at 30°C. On the third day, cultures were exposed to fluorescent light for 12 h. This culture was mixed with the infested soil at various levels. The soil was distributed in plastic boxes (9 x 9 x 10 cm) containing 500 g soil, and nine seeds of bean (*Phaseolus vulgaris* L.) were planted in each box. Disease was recorded 21 days after inoculation. Eggplant (*Solanum melongena* L.) seedlings (one day after emergence) were planted into infested soil (nine seedlings/box). Disease was recorded until damping-off ceased. Plants were grown under greenhouse conditions at 24-30°C. Disease incidence was expressed either as percentage of diseased seedlings or as disease index (Sneh *et al.*, 1966). All treatments were done in six replicates. Experiments were repeated at least twice.

Field experiments

Peanut (*Arachis hypogaea* L.) was sown under field conditions, in naturally infested alluvial soil. This experi-

ment consisted of a randomized block design and was carried out in five replicates. The plants were grown during the summer and irrigated every two weeks with a total amount of 75^3m water/ 100^2m .

Results and discussion

Utilization of carbon sources by Trichoderma harzianum

In contrast to the isolate used in a previous work (Hadar *et al.*, 1978), which attacked only *Rh. solani*, this isolate of *Trichoderma* was capable of degrading cell walls of both *Rh. solani* and *S. rolfsii*. All compounds tested known to be components of fungal cell walls (Bartnicki-Garcia, 1973) could be utilized by *T. harzianum* (Table 1).

TABLE 1

Utilization by Trichoderma harzianum of different carbon sources for growth and sporulation

| Carbon source 2mg/ml | Colony diameter after 72 h, (mm) | No. of conidia/ plate ($\times 10^6$) |
|-------------------------|-------------------------------------|--|
| Glucose | 76 | 198 |
| Laminarin | 64 | 225 |
| Chitin | 51 | 8 |
| Protein | 55 | 28 |
| Cellulose | 55 | 8 |
| None | 12 | 0 |

Of these, laminarin (β -1,3-glucan) was found most suitable for growth. Indeed, in a previous study, it was found that *T. harzianum* released highly active β -(1,3)-glucanase and chitinase into the growth medium (Hadar *et al.*, 1978).

Greenhouse experiments with artificially infested soil

When the wheat bran preparation was applied to various soils artificially infested with *Rh. solani* and planted with eggplant seedlings, a pronounced reduction of about 50% in disease incidence was observed (Table 2).

A positive correlation between the amount of *Trichoderma* preparation and disease reduction was observed in all experiments. Similar results were also obtained with *S. rolfsii* in bean seedlings (see Figure 1). A high disease incidence was observed 17 days after sowing when plants were grown in the non-treated soil, while at both concentrations of the *Trichoderma* preparation used, a significant

TABLE 2

The effect of *Trichoderma harzianum* preparation (1.5 g/kg soil) on disease incidence caused by *Rhizoctonia solani* in eggplant seedlings in various soils

| Experiment No | diseased plants (%) in soil infested with <i>Rh. solani</i> | diseased plants (%) in soil amended with <i>T. harzianum</i> preparation |
|---------------|---|--|
| 1 | 28 a* | 13 b |
| 2 | 39 a | 19 b |
| 3 | 69 a | 36 b |
| 4 | 81 a | 41 b |

* Within each line, values followed by a common letter are not significantly different ($P = 0.05$) using Duncan's multiple range test. reduction in disease incidence was observed.

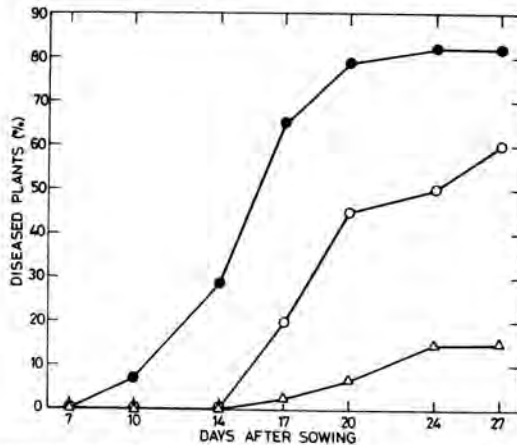


Fig. 1 Development of damping-off incidence in bean seedlings in soil infested with *Sclerotium rolfsii* (●) compared with seedlings in infested soil amended with *Trichoderma* preparation: ○ - 1.0 g/kg; △ - 3 g/kg. Treatments were significantly different ($P = 0.05$) from the control from the 14th day, using Duncan's multiple range test.

Greenhouse experiments with naturally infested soil

The same *Trichoderma* preparation was also used in a soil naturally infested with both *Rh. solani* and *S. rolfsii*, using bean seedlings as test plants. Again, a significant control was achieved with both diseases (Table 3).

TABLE 3

Biological control of bean diseases caused by Sclerotium rolfsii and Rhizoctonia solani in a naturally infested soil

| Treatment | <i>S. rolfsii</i> | <i>Rh. solani</i> | disease index ^x |
|--|----------------------|----------------------|----------------------------|
| | diseased plants, (%) | diseased plants, (%) | |
| Control | 32 a ^y | 30 a | 0.78 a |
| <i>Trichoderma</i> preparation (3 g/kg soil) | 11 b | 9 b | 0.18 b |

^x Disease index: 0 for healthy plants, 5 for killed plants.

^y Within each column, values followed by the same letter are not significantly different ($p = 0.05$) using Duncan's multiple range test.

Combined biological and chemical control

An integrated control of *Sclerotium rolfsii* was tried by using a combination of *T. harzianum* preparation and the fungicide pentachloronitrobenzene (PCNB) at low dosages. The results (Table 4) indicate a synergistic interaction between both treatments since the control achieved in the combined treatment was greater than the sum of the different treatments.

TABLE 4

Integrated control of Sclerotium rolfsii in bean seedlings by a combination of Trichoderma harzianum and PCNB

| PCNB concentration (mg/kg soil) | diseased plants (%) in soil infested with <i>S. rolfsii</i> | diseased plants (%) in soil infested with <i>S. rolfsii</i> + <i>T. harzianum</i> (3 g/kg soil) |
|---------------------------------|---|---|
| 0 | 98 a ^x | 36 b |
| 5 | 93 a | 9 c |
| 10 | 85 a | 10 c |

^x Values followed by a common letter are not significantly different ($P = 0.05$) using Duncan's multiple range test.

These results indicate that integrated control of soil-borne pathogens is possible, thus minimizing soil pollution and interference in biological balance (Papavizas, 1973; Baker and Cook, 1974; Henis and Chet, 1975).

Field experiment

The results obtained under greenhouse conditions encouraged us to test the efficiency of the *Trichoderma* preparation under field conditions. The wheat-bran preparation of *T. harzianum* was applied along with peanut seeds at a concentration of 15 g preparation/m². The results are summarized in Table 5.

TABLE 5
*Biological control of S. rolfsii in peanut plants
in a naturally infested field soil*

| Treatment | diseased plants (%) | Disease index. |
|--------------------------------|---------------------|----------------|
| Control | 56.3 a | 1.57 a |
| <i>Trichoderma</i> preparation | 43.1 b | 1.06 b |

Within each column, values followed by the same letter are not significantly different ($P = 0.05$) using Duncan's multiple range test.

From the data obtained under greenhouse and field conditions it appears that *T. harzianum* preparation is effective in controlling *S. rolfsii* and *Rh. solani* in both artificially and naturally infested soil.

These results are similar to those reported by Wells *et al.* (1972) and Backman and Rodri ues-Kabana (1975) using for biological control ryegrass and molasses as a food base for *T. harzianum*, respectively, whereas we have followed Hadar *et al.* (1978) using wheat-bran.

However, whereas in the former study (Hadar *et al.*, 1978) we have reported on an isolate which attacks only *Rh. solani* under greenhouse conditions, the isolate of *T. harzianum* used in the present study is capable of controlling both *Rh. solani* and *S. rolfsii*. The biochemical basis of this difference in specificity is not known yet and is under investigation.

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