Plant and Soil 94, 163–170 (1986). Ms. 6416

Evaluation of *Trichoderma* as a biocontrol agent for *Rosellinia necatrix*

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Received 22 May 1985. Accepted August 1985

Key words Almond Avocado Biological control *Dematophora necatrix*

Summary Different isolates of *Trichoderma harzianum* and *Trichoderma hamatum* were isolated from naturally infected roots of *Rosellinia necatrix*. *T. harzianum* and *T. hamatum* isolates overgrew cultures of *R. necatrix* placed simultaneously in petri dishes. Relative levels of *R. necatrix* population were assessed by the avocado leaf colonization method. *T. harzianum* isolate T-8, significantly reduced *R. necatrix* colonization of avocado leaves in comparison to other isolates in artificially and naturally infested soils. *R. necatrix* inoculum decreased steadily over 4 and 6 week periods in artificially inoculated soil and naturally infested soil respectively when isolate T-8 was applied, to a point where no *R. necatrix* could be recovered. Disease incidence of almond seedlings was significantly reduced when isolate T-8 was added to naturally infested En-Zurim soil.

Introduction

Rosellinia necatrix Prill. (anamorph: *Dematophora necatrix* Hartig) is the cause of white root rot disease, inflicting severe damage to fruit trees, including apple, pear, almond, peach, plum, avocado[10].

Trichoderma species are antagonists of many soilborne pathogenic fungi and have significantly decreased disease infection. Successful control of *Rhizoctonia solani* and *Sclerotium rolfsii* in greenhouse and under field conditions[1,3], control of sclerotia of *Sclerotinia sclerotiorum*[5] and control of Fusarium wilt[7] has been achieved.

Much research has been conducted using *Trichoderma* species as a natural biocontrol agent over the past decade and this study was carried out to evaluate Trichoderma as a control agent of *R. necatrix*. As of this date, to our knowledge no work has been published on biological control of *R. necatrix*.

Materials and methods

Source of Trichoderma *isolates and* R. necatrix *inoculum*

Different isolates of the antagonistic fungus identified as *Trichoderma harzianum* and *Trichoderma hamatum*[8], were isolated from apple and avocado roots infected by *Rosellinia necatrix* from the south (Kibbutz En-Zurim) and north (Kibbutz Hanita) of Israel respectively. *T. hamatum* isolates were listed as T-22, T-28, T-EZ with *T. harzianum* isolate listed

as T-8. An additional *T. harzianum* T-Y isolate, (courtesy of I. Chet) with the ability of controlling *Sclerotium rolfsii* and *Rhizoctonia solani*[3], was also used.

Production of inhibitory substances by Trichoderma *isolates*

Trichoderma isolates and *R. necatrix* were grown separately on cellophane membranes, placed on Malt Extract Agar (MEA) in petri dishes. The membranes were removed after 48 hours and a fungal disc (9 mm diameter) of *R. necatrix* was placed in the centre of each plate. Linear growth was determined every 24 hours. Fungal discs (9 mm diameter) of *Trichoderma* and *R. necatrix* were both placed on opposite ends of the same petri dish and the growth of each fungus was observed every 24 hours.

Preparation of R. necatrix *inoculum*

Wheat seeds were soaked for 12 h in water in 250 ml erlenmyer flasks each containing 100 ml seeds and subsequently autoclaved after excess water had been drained off. After sterilization of the flasks and contents, three fungal disks of two weeks old *R. necatrix* culture were placed asseptically in each flask. The flasks were incubated at 25°C and hand-shaken every two to three days to avoid clusters for a period of 14 days. The inoculum was then macerated in a Waring blendor under sterile conditions and 0.25, 0.5, 0.75, 1.0 and 1.5 g quantities were hand-mixed with sterile autoclaved soil (En-Zurim, heavy loam, pH 7.6) to determine percentage of avocado leaf disc colonization. *Trichoderma* isolate T-8, was evaluated as a biocontrol agent of *R. necatrix* at a concentration of 4.0 g wheat preparation/kg autoclaved soil.

Preparation of Trichoderma *inoculum*

The *Trichoderma* isolates were grown on a wheat-bran/peat (1:1, v/v) preparation[9] and two weeks later 1, 2, 5, and 10 g *Trichoderma* preparations were hand-mixed per kg of sterile autoclaved soil or naturally infested En-Zurim soil. A 1 g *Trichoderma* preparation per kg soil was equivalent to 10^6 spores/g of soil.

Determination of R. necatrix *inoculum level*

The avocado leaf colonization method was used to determine *R. necatrix* inoculum[11]. *R. necatrix* and *Trichoderma* preparations were hand-mixed with 1 kg soil and distributed in 4 plastic containers (11 by 11 by 4 cm) each holding 250 g soil, with avocado leaf discs (1.6 cm diameter) acting as traps for *R. necatrix*. These containers were incubated at 25°C for 12–14 days after which disc colonization was assessed in comparison to controls. All treatments were carried out in 4 replicates each bearing 15 avocado discs. Soil treatments in all experiments were set in randomized blocks and repeated at least twice. Statistical significance of the data was determined by analysis of variance, $P = 0.05$.

Greenhouse experiments

T. harzianum isolate T-8, was hand-mixed with naturally infested soil and distributed in plastic plant plots (7 cm diameter) each holding 200 g soil. Two weeks after germination, almond seedlings var. Umm-el-Fahm, were planted to detect disease incidence under greenhouse conditions of 23 ± 2°C and daily unmonitored water supply. Disease incidence was expressed as percentage of killed plants. All treatments were carried out at 10 replicates having a single plant per replicate. The experiment was terminated after 20–24 weeks and repeated twice in En-Zurim soil and once in another naturally infested soil.

Results

Antagonism between R. necatrix *and* Trichoderma *isolates*

Linear growth of *R. necatrix* was not inhibited after growing on MEA plates where *T. harzianum* and *T. hamatum* were previously

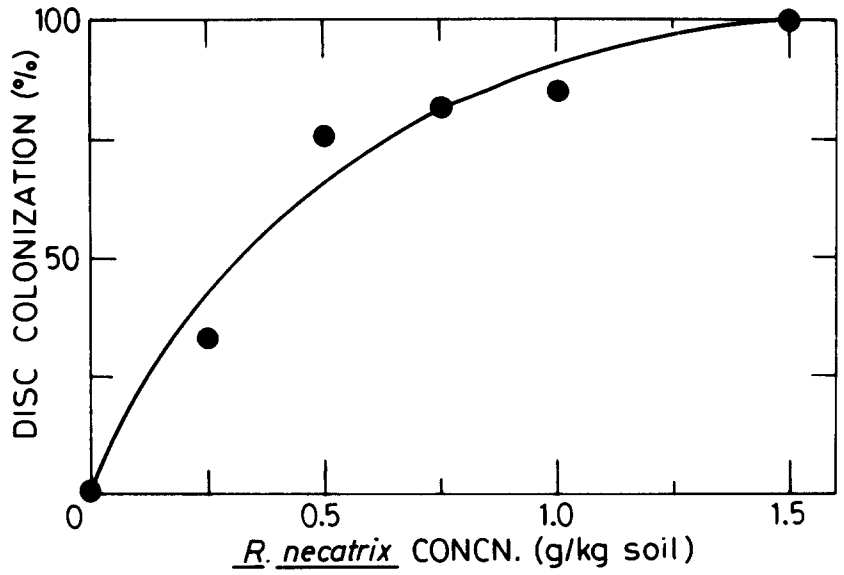

Fig. 1. Avocado leaf disc colonization (%) by artificially inoculated *Rosellina necatrix* (g preparation per kg uninfested soil).

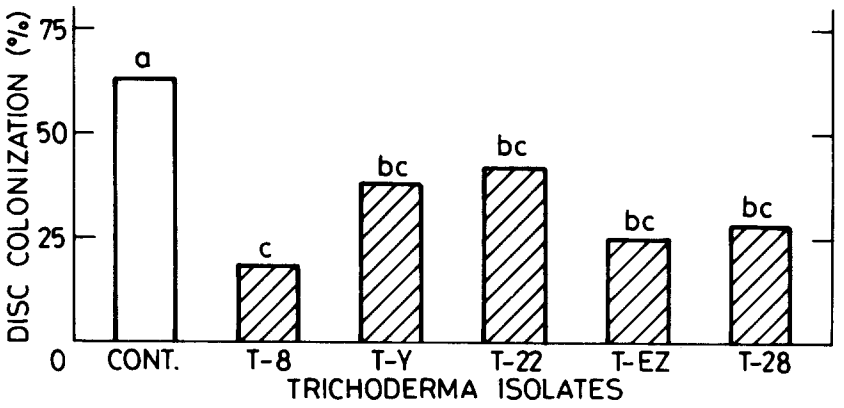

Fig. 2. Avocado leaf disc colonization (%) by *Rosellinia necatrix* with different isolates of *Trichoderma* in naturally infested soil (5 g preparations per kg naturally infested soil). Control (cont.) – naturally infested soil. Columns having a *common letter* are not significantly different ($P = 0.05$).

grown in comparison to a control. However, when placed one opposite the other on MEA plates mycelial growth of the *Trichoderma* isolates proceeded to cover the whole culture of *R. necatrix* with various degrees of sporulation.

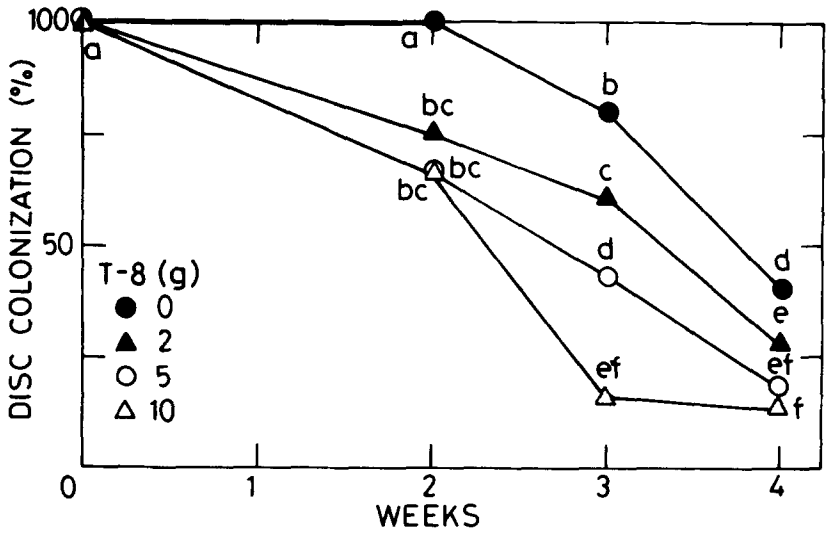

Fig. 3. Inoculum decrease of *Rosellina necatrix* in artificially inoculated autoclaved soil (4 g preparation/kg soil) with increasing concentrations (0, 2, 5, 10 g preparation/kg soil) of *Trichoderma harzianum* isolate T-8 over a 4 week period. Values having a *common letter* are not significantly different ($P = 0.05$).

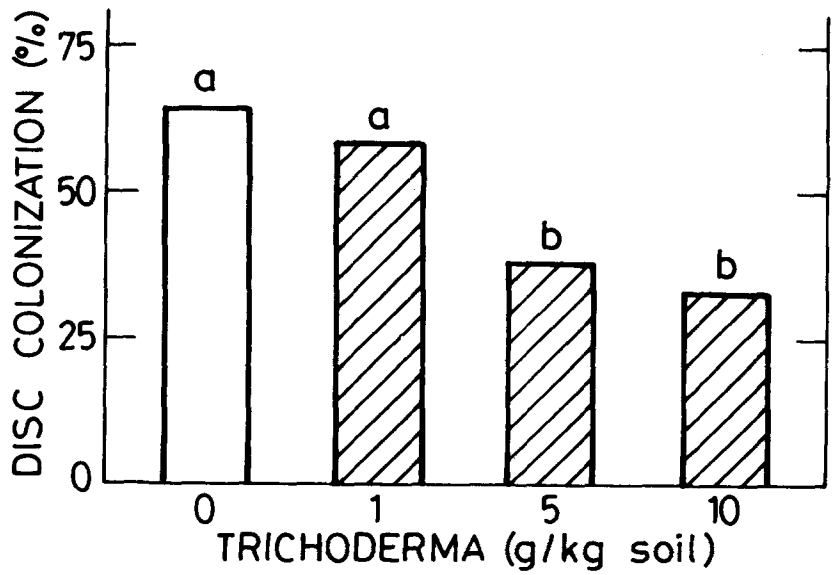

Fig. 4. Effect of different concentrations (0, 1, 5, 10 g preparation/kg soil) of *Trichoderma harzianum* isolate T-8 on disc colonization by *Rosellinia necatrix* in naturally infested soil. Columns having a *common letter* are not significantly different ($P = 0.05$).

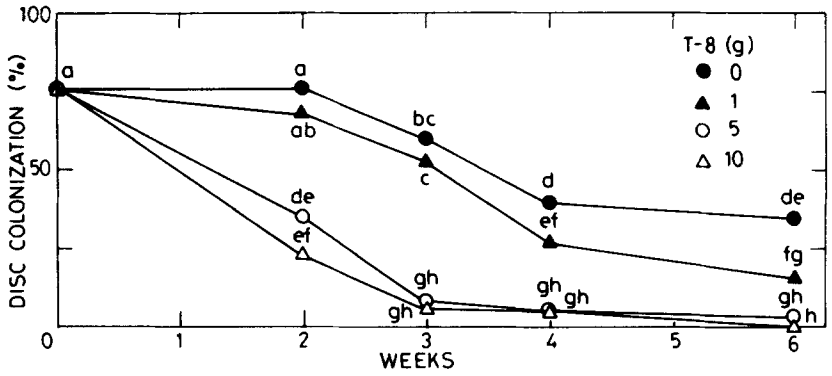

Fig. 5. Inoculum decrease of *Rosellinia necatrix* in naturally infested En-Zurim soil with increasing concentrations of *Trichoderma harzianum* isolate T-8 (0, 1, 5, 10 g/kg soil) over a 6 week period. Values having a *common letter* are not significantly different ($P = 0.05$).

Screening and evaluation of Trichoderma *isolates in artificially and naturally infested* R. necatrix *soils*

The avocado leaf colonization method was reliable for assessing relative levels of the pathogen population and within certain limits, inoculum density and rate of colonization were closely related (Fig. 1).

In artificially inoculated soil isolate T-8 significantly reduced % disc colonization at 5 g and 10 g preparations in comparison to the control. Percentage of leaf colonization of other isolates were either not significantly different from the control or of a higher value in comparison to isolate T-8.

In naturally infested soil isolate T-8 significantly reduced avocado leaf colonization by 45% in comparison to isolates T-Y, T-22, T-EZ and T-28 by 29%, 25%, 38% and 35% respectively (Fig. 2). Following these results we proceeded to use T-8 exclusively in further experiments.

Preparations of isolate T-8 at 2, 5 and 10 g concentrations significantly reduced *R. necatrix* disc colonization over all of the 2, 3 and 4 week periods in comparison to the control in artificially inoculated soil. Reduced leaf colonization resulted with increasing concentrations of *T. harzianum* preparation (Fig. 3).

Biological control of R. necatrix *in naturally infested soils*

R. necatrix in naturally infested En-Zurim soil colonized discs by 64% with a significant reduction resulting from applications of 5 g and 10 g *T. harzianum* isolate T-8 preparations by 26 and 31% respectively (Fig. 4). In another naturally infested soil inoculum density

had to be diluted in order to achieve similar results. Over a six week period *R. necatrix* inoculum in naturally infested En-Zurim soil decreased steadily in accordance with the disc colonization method (Fig. 5). Treated soil significantly reduced disc colonization over the 6 week period and the disease was not detected at all with a 10 g preparation of isolate T-8 6 weeks later in comparison to a 35% colonization by the control (Fig. 5). The 5 g preparation reduced colonization to 2%.

Greenhouse experiments

Disease incidence in two separate experiments of 75% and 60% in almond seedlings was significantly reduced to 20% and 20% when a 5 g preparation of isolate T-8 was added to naturally infested En-Zurim soil (Fig. 6). In a similar experiment using another naturally infested soil disease mortality of almond seedlings was reduced by 20% from 70% in naturally infested soil to 50% in treated soil (5 g preparation of isolate T-8).

Discussion

Trichoderma harzianum isolate T-8, proved to be effective in controlling *Rosellina necatrix*. Other isolates of *T. harzianum* and *T. hamatum* were less efficient and isolate T-Y which specializes in controlling *S. rolfsii* and *R. solani* was effective but less so than isolate T-8[3]. Also noted by the same authors is the fact that in culture *T. harzianum* isolate T-Y, did not possess antagonistic potential against *R. necatrix*.

In this work no inhibitory substances were detected in hindering the pathogen, where as one isolate of *T. harzianum* was found to significantly inhibit *Pythium aphanidermatum*[9]. We can thus assume that the antagonistic isolate T-8 either competes more successfully for food substrate or behaves as a mycoparasite on the pathogen possessing lytic enzymes[2,6].

In both artificially inoculated and naturally infested soils, T-8 isolate reduced *R. necatrix* populations over a 4–6 week time period which shows a continuous effect of the antagonist eventually disposing entirely of *R. necatrix* when applied at high concentrations. Increased death rate of *R. solani* in time with successive plantings of radish seedlings in soil amended with *T. harzianum* was similarly achieved[4].

Field trials utilizing *T. harzianum* to control *R. necatrix* in fruit groves are being presently conducted and numerous applications of

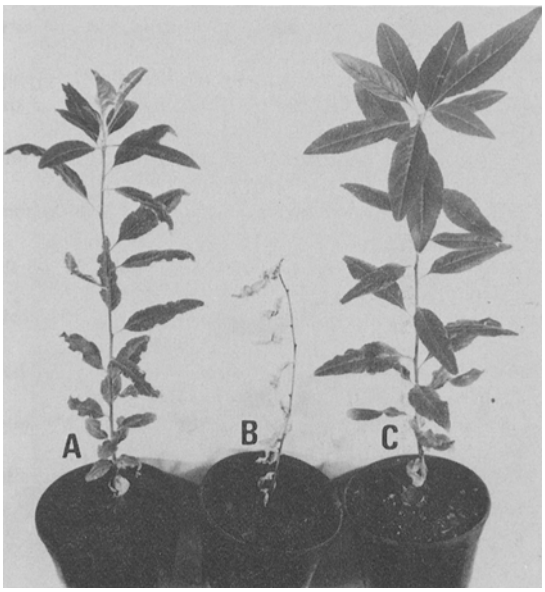

Fig. 6. Disease incidence in almond seedlings planted in naturally infested soil (One sample of the 10 replicates).
A – non-infested soil.
B – naturally infested soil.
C – 5 g preparation of *Trichoderma harzianum* isolate T-8 per kg naturally infested soil.

T. harzianum in the field should be necessary to control *R. necatrix* under these conditions.

No efficient measures of control of *R. necatrix* in an infected established orchard have been reported. To date no biological agent has been used to control *R. necatrix* in an established grove of fruit trees but with the application of a biological agent a possible reduction of inoculum and further spread of *R. necatrix* may be achieved under these conditions.

Acknowledgements This work was partially supported by a grant from the United States – Israel Binational Agricultural Research and Development Fund (BARD) and by a grant from the Fruit Production and Marketing Board, Tel Aviv, Israel.

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