Biological Control of *Rhizoctonia solani* by *Trichoderma harzianum* in Carnation

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ABSTRACT

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Wheat bran culture of *Trichoderma harzianum* was tested for control of *Rhizoctonia solani* in carnation in fields treated with methyl bromide. A linear correlation was obtained between rate of *T. harzianum* preparation applied to soil and degree of disease control. Disease incidence was reduced 70% when the *T. harzianum* preparation was applied at 150 g (dry wt) per square meter. *T. harzianum* gave best disease control when applied and established in the rooting mixture before transplanting in the field. This method was superior to the broadcast application because it required lower rates of application.

Additional key words: biocontrol, pentachloronitrobenzene, soilborne pathogens, stem rot

Rhizoctonia solani (Kuehn) causes stem rot on carnation (*Dianthus caryophyllus* L.) during the hot season (7). The recommended method for controlling the pathogen in Israel is soil fumigation with methyl bromide, followed by incorporation with a rake of pentachloronitrobenzene (PCNB) at 70–80 kg/ha (8). The soil fumigation is designed to control other soilborne pests (such as *Fusarium* and nematodes), and it is not successful in controlling *R. solani*.

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Isolates of *Trichoderma* sp. are antagonistic to several pathogenic fungi (2,10). *T. harzianum* as a food base was successfully used to control *Sclerotium rolfsii* (1,12). A wheat bran culture of *T. harzianum* was recently used as a biocontrol of both *R. solani* and *S. rolfsii* under field conditions in several crops (3–6,9).

This study tested the ability of *T. harzianum* to control stem rot of carnation caused by *R. solani* under field conditions.

MATERIALS AND METHODS

Two field experiments were conducted with carnations grown in central Israel. The sandy loam soil consisted of 81.8% sand, 2.3% silt, 15.4% clay, and 0.5% organic matter; pH was 7.4 and moisture-holding capacity was 12.2%. Both experiments were conducted in six replicates arranged in a randomized block design. Individual plots were 2 m long and 1 m wide, with four rows of 17 plants each. Soil in the test area was fumigated with a compound containing 98% methyl bromide 3 wk before planting.

R. solani was isolated from diseased carnation plants. We prepared chopped potato soil inoculum from this isolate according to Ko and Hora (11) by growing the pathogen in an autoclaved mixture of potato and soil (1:4, w/w) for 20 days. One week after fumigation, the inoculum was incorporated into the soil with a rotary hoe to a depth of 10 cm.

An isolate of *T. harzianum* Rifai aggr. capable of parasitizing *R. solani* (3–6,9) was grown on autoclaved wheat bran for 2 wk. Three days before planting, this preparation was broadcast on the row surface and incorporated into the soil to a depth of 7 cm by a rotary hoe. In the 1978 experiment, we compared the broadcast application of the preparation at 150 g (dry wt) per square meter with the conventional PCNB treatment and an untreated control. In 1979, we also broadcast lower rates (50 and 100 g/m^2) of the preparation.

Carnation (*D. caryophyllus* 'Red Baron') was planted on 16 July 1978 in the first experiment and 17 June 1979 in the second one. *R. solani* only attacks carnation during the first 5 mo of growth. Low temperatures prevent disease development during the following 6 mo, which is also the time of flower cutting.

Plants showing typical symptoms of *R. solani* stem rot during the growth period were uprooted and examined. We verified the presence of the pathogen by plating plant tissues on 2% tap water agar containing chloramphenicol at 250 μg/g Results were expressed as percentage of diseased plants.

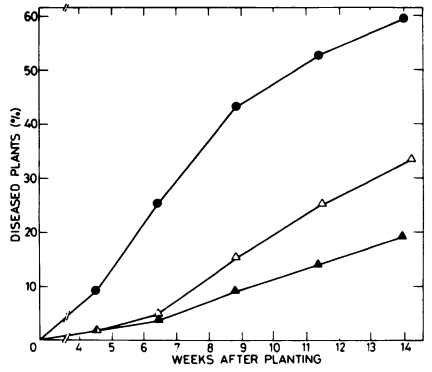

Fig. 1. Effect of 1978 treatments on incidence of disease caused by *Rhizoctonia solani* in carnation. ▲ = *Trichoderma harzianum* preparation applied to soil at 150 g/m^2; Δ = pentachloronitrobenzene applied at 10 g/m^2; ● = untreated control.

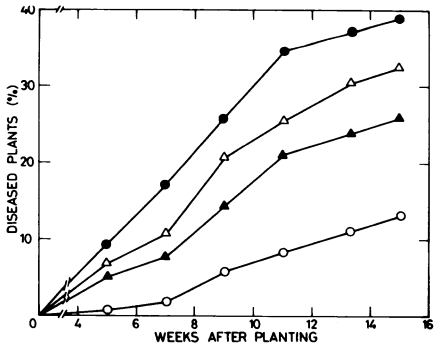

Fig. 2. Effect of 1979 treatments on incidence of disease caused by *Rhizoctonia solani* in carnation. Δ = *Trichoderma harzianum* preparation applied to soil at 50 g/m^2; ▲ = *T. harzianum* preparation applied at 100 g/m^2; o = *T. harzianum* preparation applied to rooting mixture (15% by volume); ● = untreated control.

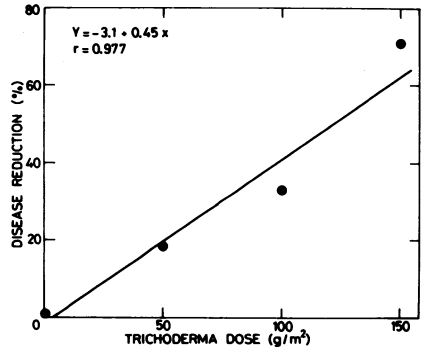

Fig. 3. Correlation between application rate of *Trichoderma harzianum* and control of disease caused by *Rhizoctonia solani* in carnation.

We also tested the effectiveness of adding *T. harzianum* directly to the rooting mixture. Carnation cuttings were rooted by the Speedling method. The rooting mixture was peat alone or amended with 15% by volume of *T. harzianum* preparation for application to the root zone. The mixture was put into Speedling-type, preformed trays consisting of 168 conical compartments (3 × 5 × 10 cm), into each of which a carnation cutting was rooted. Three weeks later, the seedlings were transplanted together with the rooting mixture.

On transplanting day in 1978, a compound containing 75% PCNB was sprayed on the row at 10 g/m^2 and incorporated into the soil by a rake to a depth of 7 cm.

The fields used in these experiments were fertilized before planting with superphosphate at 2,000 kg/ha and potassium chloride at 500 kg/ha. During the growth period, potassium nitrate and ammonium sulphate were applied weekly, totaling up to 6,000 kg/ha each. Plants were irrigated and treated with pesticides according to standard practice in this region.

Soil samples were tested for density of *Trichoderma* propagules using the soil dilution method on *Trichoderma*-selective media. Colony-forming units (CFU) were counted 3–4 days later.

RESULTS AND DISCUSSION

Broadcast application of *Trichoderma*. In 1978, a wheat bran preparation of *T. harzianum* was applied to field plots at 150 g/m^2. Diseased plants appeared in the untreated control plots 2 wk after transplanting; disease incidence increased to 9% at 4 wk and 59% at 14 wk. Disease incidence was significantly less in plots treated with *T. harzianum* and PCNB, occurring in 2% of plants in both plots 4 wk after planting and in 19 and 33% of plants treated with *T. harzianum* and PCNB, respectively, 10 wk after planting (Fig. 1). Flower yield was 107, 137, and 161 flowers per square meter in the control, PCNB, and *T. harzianum* treatments, respectively.

In 1979, the *T. harzianum* preparation was applied at lower rates of 50 and 100 g/m^2. Disease incidence caused by *R. solani* in the untreated control was similar to that in the former experiment, and reached 39% by 15 wk after transplanting. At 50 g/m^2, the *T. harzianum* preparation only reduced disease incidence to 32%, which was not statistically different from the control ($P = 0.05$); at 100 g/m^2, the *T. harzianum* preparation reduced disease incidence to 26%, which was significantly different ($P = 0.05$) (Fig. 2).

We compared the data from the different experiments by calculating the percentage of reduced disease incidence relative to the untreated control. A linear correlation was obtained between dosage of *T. harzianum* and percentage of disease reduction ($R = 0.977$) (Fig. 3). We concluded that satisfactory disease control could be achieved only by a relatively high dose of *T. harzianum* preparation.

According to soil samples, the natural *Trichoderma* population in the control plot was low. Although population density in the treated plots varied according to the application rate of *Trichoderma* preparation (Table 1), it declined in time to the level of 10^3–10^5 CFU/g of soil. When soil samples from the 1978 experiment were assayed one year after planting, the *Trichoderma* population density was 1.1×10^4 and 4.5×10^2 CFU/g of soil in *Trichoderma*-treated and control plots, respectively.

Application to rooting mixture. When *Trichoderma* preparation was applied to the soil, the antagonist had no direct contact with the root zone because it was covered with peat. We introduced the biological control agent into the root zone by rooting carnation plants as Speedlings in peat supplemented with *T. harzianum* preparation (15% by volume). When transferred to soil infested with *R. solani*, the treated plants had the lowest infection rate (13% diseased plants) obtained—significantly lower even than when *T. harzianum* was broadcast (Fig. 2). Disease incidence was not further reduced by combining the rooting mixture and broadcast methods of *Trichoderma* application.

Application of *T. harzianum* preparation to the carnation root system before rooting in the nursery had several advantages. The antagonist was established and allowed to multiply in the root system without causing damage to the plant and while protecting the root collar. The total amount of preparation used was extremely low: 13.5 g/m^2 (34 plants per square meter) as compared with the 150 g/m^2 used in the infested plot to obtain similar control.

In this study, *T. harzianum* provided effective biocontrol of *R. solani* in

Table 1. Density of *Trichoderma harzianum* population in soil treated at various rates[a]

Weeks after application	Density (CFU/g of soil) at application rate (g/m^2) of			
	0	50	100	150
0	0.5×10^2	6.7×10^4	1.2×10^5	4.6×10^8
10	1.0×10^2	8.7×10^3	5.3×10^4	1.7×10^6
14	4.0×10^2	9.5×10^3	2.1×10^4	5.5×10^5

[a]Soil samples were diluted in sterile water and spread on medium selective to *T. harzianum*. Colony-forming units (CFU) were counted 3–4 days later.

carnation. Restricted application of the agent to the root zone in the nursery makes this biological control even more attractive from a commercial point of view.

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