

## Biological Control of Fusarium Crown Rot of Tomato by *Trichoderma harzianum* Under Field Conditions

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### ABSTRACT

Sivan, A., Ucko, O., and Chet, I. 1987. Biological control of Fusarium crown rot of tomato by *Trichoderma harzianum* under field conditions. *Plant Disease* 71:587-592.

Biological control experiments were carried out during two successive growing seasons in tomato fields naturally infested with *Fusarium oxysporum* f. sp. *radicis lycopersici*. *Trichoderma harzianum* was applied as a seed coating or as a wheat-bran/peat (1:1, v/v) preparation introduced into the tomato rooting mixture. *Trichoderma*-treated transplants were better protected ( $P=0.05$ ) against Fusarium crown rot than untreated controls when planted in methyl bromide-fumigated or nonfumigated infested fields. The total yield of tomatoes in the *T. harzianum*-treated plots was increased as much as 26.2% over the controls. When *T. harzianum* was applied to the root zone of tomato transplants, it proliferated successfully in the rhizosphere. Soil samples taken from the crown area 5–10 cm from the plant stem showed an increase in *T. harzianum* population levels during the growing season; however, no significant decline was found in the soil population density of *Fusarium* spp. in the same soil samples. When tomato seeds previously treated with conidia of *T. harzianum* were sown in a naturally infested field, the antagonist was detected on root segments from plants sampled 20 wk after planting. The highest counts of the antagonist were detected on the root tips, resulting in the complete reduction of *Fusarium* spp. recovered from these segments.

Fusarium crown rot of tomatoes induced by *Fusarium oxysporum* f. sp. *radicis lycopersici* (*F. o. f. sp. radicis lycopersici*) Jarvis & Shoemaker was first reported in Japan in 1974 (27). In the same year, it was observed in greenhouses in Ohio (9) and in southern Florida (24). The disease was first identified in Israel in 1980, when it caused heavy yield losses.

Repeated attempts to control the disease by soil disinfection have failed, apparently because of airborne dispersal of microconidia of the pathogen (20). Resistant commercial cultivars are not

available (12), and at present, the only effective control measure is the application of a captafol drench to greenhouse beds immediately after steaming (19). This minimizes the reinfestation of steam-disinfested soils by the pathogen.

One significant phenomenon in biological control of Fusarium wilt diseases is the existence of suppressive soils in which microbial activity prevents the appearance of disease in susceptible crops. Alabouvette et al (2) showed that the suppression of *F. oxysporum* f. sp. *melonis* might be induced by natural saprophytic fungal communities of *F. oxysporum* and *F. solani*. Soil suppressiveness to *Fusarium* spp. was also shown in the presence of siderophore-producing pseudomonads. These microorganisms

bind  $Fe^{+3}$ , making it less available to those microflora unable to produce similar iron transport agents (13,21).

The biological control potential of *Trichoderma* spp. against soilborne plant pathogens is well known (5,6,10,11,18, 22,23,25); however, few data are available showing control of Fusarium wilt pathogens by *Trichoderma* spp. Marois et al (15) showed the potential of a multifungus conidial suspension including *T. harzianum* in the control of tomato crown rot when tested in a fumigated soil. Locke et al (14) recently reported the use of *T. viride* for controlling *F. oxysporum* f. sp. *chrysanthemi* in a steamed soil mix.

Sivan and Chet (22) have recently shown the effectiveness of a newly isolated strain of *T. harzianum* in controlling Fusarium wilt of cotton and melons as well as of *F. culmorum* in wheat under natural soil conditions.

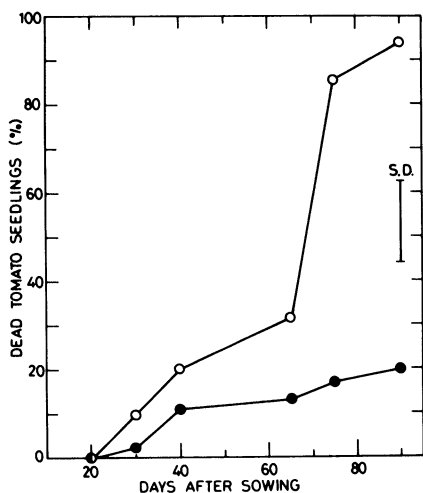
In this study, we examined the potential of this isolate to control Fusarium crown rot of tomatoes under field conditions and its presence in the rhizosphere of tomatoes after a conidial seed treatment.

### MATERIALS AND METHODS

**Fungal isolates.** An isolate of *T. harzianum* obtained from a Fusarium-wilted cotton plant and designated T-35 (22) was cultured on a synthetic medium (SM) (17) and served as the biocontrol agent during this study. *F. o. f. sp. radicis lycopersici* was isolated from a diseased

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**Fig. 1.** Biological control of *Fusarium* crown rot of tomatoes under greenhouse conditions. *Trichoderma harzianum*-coated seeds (●—●) were compared with untreated seeds (○—○). The sowing was carried out in soil artificially infested with *Fusarium oxysporum* f. sp. *radicis lycopersici*. Disease reduction at the end of the experiment was significant ( $P = 0.05$ ).

tomato plant collected from an infested field in southern Israel and cultured on a solidified yeast extract-glucose medium (YM) containing (in g/L of distilled water) yeast extract (Difco Laboratories Detroit, MI), 5; peptone (Difco), 5; glucose, 10; and agar (Difco), 20, and was incubated at 27 C.

**Greenhouse experiments.** Biological control tests were carried out in artificially infested sandy loam soil (pH 7.4) consisting of 82.3% sand, 2.3% silt, 15% clay, 0.3% organic matter, 0.02% N, 0.06% K, 0.01% P, and 0.003% extractable Fe and having a moisture (at field capacity) of 12.2%. Soil infestation with the pathogen was performed using a microconidial suspension of *F. o. f. sp. radicis lycopersici* (22) Erlenmeyer flasks (250 ml) each containing 50 ml of liquid YM and were seeded with mycelial disks from 72-hr-old cultures of the pathogen. Flasks were incubated at 27 C in a rotary shaker at 120 rpm for 4 days. Microconidia were then separated from the mycelium by filtration through eight layers of surgical gauze. The conidial suspension

was washed three times by centrifugation at 9,150 g for 30 min at 4 C. Ten milliliters of this suspension, adjusted to  $2 \times 10^7$  microconidia per milliliter, was added to sandy loam soil with an electrical soil mixer. Plastic pots, each containing 3 kg of infested soil, were sown with 10 tomato seeds.

*T. harzianum* was applied as seed coating; conidia were collected from cultures grown in Erlenmeyer flasks (24), each containing 200 ml of solidified potato-dextrose agar (Difco). The conidial suspension was adjusted to  $5 \times 10^9$  conidia per milliliter and supplemented with 0.015% (v/v) of Nu-film 17 (Miller Chemicals), which served as an adhesive. Ten grams of tomato seeds were treated with 3 ml of the conidial suspension and immediately dried by ventilation.

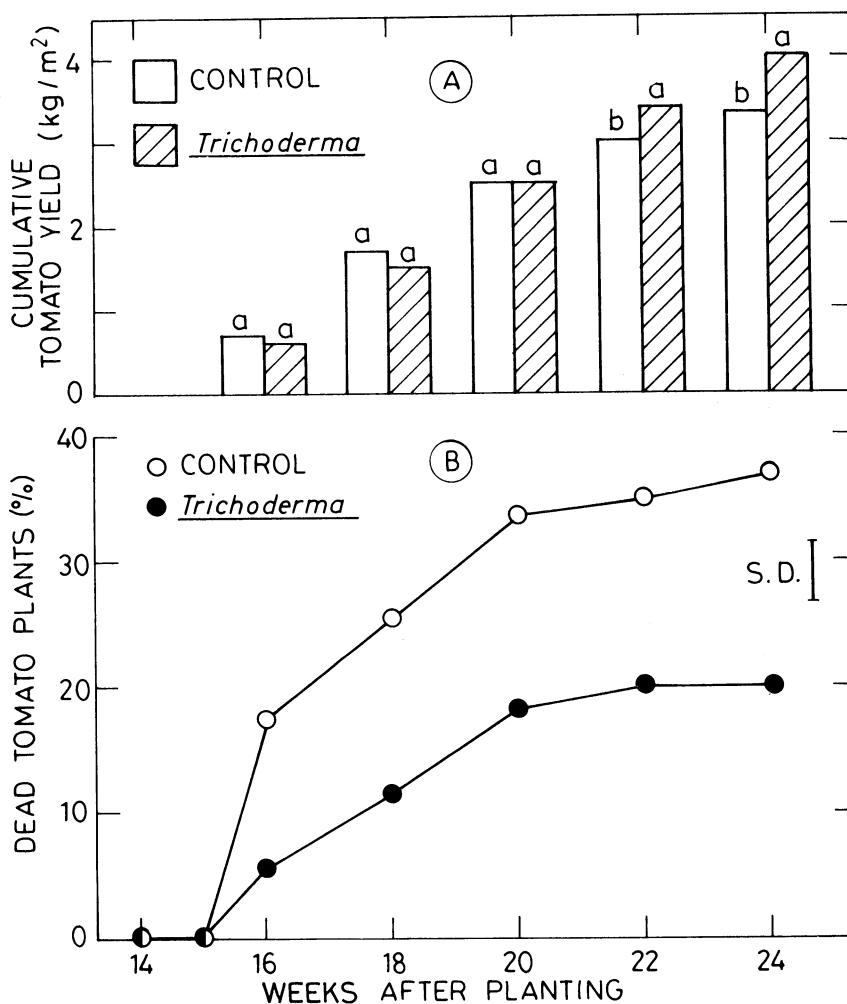
The density of *T. harzianum* conidia on the seed coat surface was determined by serial dilutions of suspensions from treated and untreated seeds, using a *Trichoderma*-selective medium (TSM [8]).

**Field experiments.** Three field experiments were carried out during the 1984 and 1985 growing seasons in naturally infested tomato fields at the Neot Hakikar Experimental Station in the Arava Valley, southern Israel. These fields were heavily damaged by this pathogen during the five previous seasons. The first and second experiments were conducted during the 1984 growing season, and the third was carried out a year later on the same field plot as field experiment 1 and involved soil fumigation with methyl bromide.

In these experiments, tomato field plots (two beds, each  $12 \times 1.8$  m) were covered with a 0.25-mm-thick plastic mulch before planting.

In field experiments 1 and 3, *T. harzianum* was applied in the nursery as a wheat-bran/peat preparation (23). Wheat-bran/peat mixture (1:1, v/v) adjusted to 40% moisture (w/w) was autoclaved in autoclavable polyethylene bags ( $50 \times 50$  cm) for 1 hr at 121 C on three successive days. The substrate mixture was inoculated with homogenized mycelium from 48-hr cultures of *T. harzianum* grown in liquid SM and incubated in an illuminated chamber for 14 days at 30 C. This preparation, containing  $5 \times 10^9$  cfu/g (dry wt), was mixed with the propagative mixture of tomatoes (peat and vermiculite, 1:1, v/v) at a concentration of 10% (v/v). Tomato (*Lycopersicon esculentum* Mill. cv. 1684-Naama) seeds were sown in Speedling-type trays consisting of 180 conical compartments each measuring  $3.7 \times 3.7 \times 10$  cm. At the end of the rooting period, 30 days after sowing, the seedlings were transplanted in the field, spaced 50 cm apart in one row per bed.

In field experiment 1, treatment A consisted of transplants treated with a *Trichoderma* preparation, whereas treatment B contained untreated trans-



**Fig. 2.** Biological control of *Fusarium* crown rot of tomatoes under field conditions (field experiment 1). *Trichoderma harzianum* was applied as a wheat-bran/peat preparation introduced to the rooting mixture of tomato transplants (10%, v/v). (A) Cumulative yield of tomatoes; bar values on each harvest date marked with the same letter are not significantly different ( $P = 0.05$ ). (B) Disease incidence; disease reduction determined 16, 18, 20, and 24 wk after planting was significant ( $P = 0.05$ ). Statistical comparisons were done by *t* test.

plants. Soil samples were taken at random from both treatments, at distances of 5–10 cm and a depth of 5 cm from the plant crown.

Field experiment 2 examined the recovery of *T. harzianum* and *F. oxysporum* from tomato roots after seed treatment with *T. harzianum*. This experiment was carried out in a naturally infested field near the first field experiment. Seeds in treatment A were coated with *T. harzianum*; seeds in treatment B were untreated. At the end of the growing season, tomato plants were carefully uprooted; the roots were dipped in water to remove the soil and dried on Whatman No. 1 chromatography paper. The roots were cut into 5-cm segments and suspended in sterile distilled water from which serial soil dilutions were plated on TSM and a *Fusarium*-selective medium (SQA [16]) for *Trichoderma* spp. and *Fusarium* spp. root populations, respectively. Results were expressed as colony-forming units per gram of root segment (fresh weight).

In field experiment 3, *T. harzianum*-treated or untreated tomato transplants were planted in unfumigated commercial field or in a field fumigated with methyl bromide. Treatment A, the control, consisted of untreated transplants, and treatment B contained transplants treated with a wheat-bran/peat preparation. In treatment C, the soil was fumigated with methyl bromide (750 kg/ha) and treatment D consisted of a combination of methyl bromide and *T. harzianum* (B + C). Cultural practices in all field experiments were similar to those employed in this region.

During the growing season, disease incidence was recorded and expressed as the percentage in a population of the completely wilted plants.

The *F. o. f. sp. radialis lycopersici* inoculum potential of soil samples taken from the field was estimated using untreated tomato seeds. Soil samples were taken every month from both treatments of field experiment 1 and were placed in plastic boxes (9 × 9 × 10 cm) containing 0.5 kg of soil in which 10 untreated tomato seeds were sown. Changes in the level of fungal populations in these samples were determined by serial soil dilutions with TSM and SQA for *Trichoderma* and *Fusarium* spp., respectively.

Greenhouse and field experiments were conducted in six replicates arranged in a randomized block design. Greenhouse experiments were repeated at least twice. Results of all tests were statistically analyzed using *t* test or linear regressions at *P* = 0.05.

## RESULTS

When *T. harzianum* was applied as a seed coating (Fig. 1), the crown rot incidence of greenhouse-grown tomatoes was reduced up to 80% by 75 days after sowing.

Under field conditions (field experiment 1), application of the antagonist as a wheat-bran/peat preparation to the propagative mixture of tomato transplants (Fig. 2B) resulted in a significant (*P* = 0.05) reduction of disease incidence throughout the growing season. The cumulative yield of tomatoes was significantly (*P* = 0.05) increased by 18.8% (Fig. 2A).

Field experiment 3 was performed a year later on the same field plot as field experiment 1. Results of these successive experiments (Fig. 2, Table 1) showed a remarkable increase in disease incidence in the untreated plots, where an increase from 37% in the first year to 74% in the second year was noted. When applied to unfumigated plots, *T. harzianum* reduced the incidence of crown rot in the two successive seasons. The best disease control in the third field experiment was obtained by the combined treatment of methyl bromide and *T. harzianum* (87% disease reduction). Application of *T. harzianum* to the root zone of tomato transplants increased yields only in the first year (Fig. 2). In the second year, *T. harzianum* increased tomato yields only when integrated with soil fumigation. This combination of fumigant with antagonist had the maximal effect on the total and on the "class 1" tomato yields (26.2 and 41.8% yield increases, respectively).

The inoculum potential of *F. o. f. sp. radialis lycopersici* was evaluated in the greenhouse with soil samples taken near the crown area of tomatoes from the field (Fig. 3). Sowing untreated seeds in soil samples taken from *T. harzianum*-treated plots resulted in a lower incidence of disease than that observed in plants grown in samples taken from the untreated plots (Fig. 3A). No significant difference in *Fusarium* spp. populations was found between samples taken during the season from *Trichoderma*-treated plots and those taken from the control (Fig. 3B). *T. harzianum*, when applied as a wheat-bran/peat preparation to the rooting mixture of tomatoes, proliferated in the root zone and its population density increased from  $7 \times 10^6$  cfu/g of peat adhered to roots at planting to  $6.6 \times$

$10^2$  cfu/g 20 wk later. The levels of *Trichoderma* spp. soil populations in samples of treated plots increased from 100 on the planting date to 740 cfu/g of soil 24 wk after planting (Fig. 3C). In contrast, the population density of the antagonist in the control plots decreased by the end of the season to fewer than 10 propagules per gram of soil (Fig. 3C).

When *T. harzianum* was applied as a seed coating in a field naturally infested with *F. o. f. sp. radialis lycopersici*, it was readily isolated from tomato roots (Fig. 4A) 20 wk after sowing. The highest recovery of *T. harzianum* from root segments was recorded on segments close to the stem crown and those including the root tips. These contained 26 and 36 cfu/g of root, respectively; however, the total population density of *Trichoderma* recorded on the root surface was lower than that found on the tomato seed coat ( $2 \times 10^6$  cfu/g of seed). The recovery of *Fusarium* spp. from tomato roots (Fig. 4B) indicated that it was reduced mainly on those segments showing the highest density of *T. harzianum*. Complete elimination of *Fusarium* was obtained on segments that included root tips.

## DISCUSSION

*T. harzianum* was effective in reducing the incidence of *Fusarium* crown rot of tomato under field conditions throughout two successive growing seasons.

In the past, application of the same isolate of *T. harzianum* under greenhouse conditions resulted in significant control of *F. oxysporum* on cotton and melon and of *F. culmorum* on wheat (22). Marois et al (15) were the first to demonstrate successful biological control of *Fusarium* crown rot on tomatoes with *T. harzianum*. However, they applied a conidial suspension consisting of three isolates of *T. harzianum*, one of *Penicillium fusiculosum*, and one of *Aspergillus ochraceus* to an infested field fumigated with methyl bromide. Those antagonists reduced disease incidence but had no effect on tomato yield. In this study, the beneficial antagonistic effect of *T. harzianum* on both disease incidence and tomato yield was demonstrated.

**Table 1.** Effects of integrating *Trichoderma harzianum* and methyl bromide on crown rot incidence and on tomato yield under field conditions

| Methyl bromide dose (kg/ha) | <i>Trichoderma</i> preparation <sup>x</sup> | Disease incidence <sup>y</sup> (%) | Yield (t/ha) |        |
|-----------------------------|---|------------------------------------|--------------|--------|
|                             |   |                                    | Class 1      | Total  |
| 0                           | Not applied                                 | 74.0 a <sup>z</sup>                | 19.7 c       | 33.2 c |
| 0                           | Applied                                     | 52.0 b                             | 21.9 c       | 35.6 c |
| 750                         | Not applied                                 | 23.5 c                             | 35.4 b       | 58.6 b |
| 750                         | Applied                                     | 9.5 d                              | 50.2 a       | 74.0 a |

<sup>x</sup>Wheat-bran/peat preparation (1:1, v/v) was applied (10%, v/v) to the rooting mixture of tomato seedlings in the nursery.

<sup>y</sup>Plants showing partial or complete wilt symptoms. In these plants, the pathogen could be easily isolated from the crown after external disinfestation with 10% sodium hypochlorite.

<sup>z</sup>Numbers in each column followed by the same letter are not significantly different (*P* = 0.05) according to Duncan's multiple range test.

Moreover, in field experiment 1, these effects were obtained by application of the biocontrol agent even in the absence of a prebiocidal treatment.

The field experiments in this study were conducted under a higher inoculum pressure, resulting in a rapid increase in the total disease incidence of tomatoes planted in the untreated plots, from 37% in the first year to 74% in the second year. Under these conditions, application of

the biocontrol agent alone significantly reduced disease incidence in both growing seasons. *Trichoderma* treatment in the first growing season had a beneficial effect on tomato yields that lead to an 18.8% increase. In the second year, however, the antagonist was found to improve yields only when combined with methyl bromide. This failure of the antagonist, when applied alone in the second season, to improve yields might

be explained by the higher inoculum pressure compared with the first year.

It was recently shown that *T. harzianum* prevented *Rhizoctonia solani* from reinfesting soils fumigated with methyl bromide (25). In our study, application of *T. harzianum* to the root zone of tomatoes significantly improved the efficacy of methyl bromide. An integrated treatment with both the biocontrol agent and the fumigant was most effective in reducing disease incidence. An 87% reduction in disease incidence and a significant 41.8% increase of "class 1" tomato yields were achieved.

When the antagonist was introduced into the root zone of tomato transplants, it became established in the rhizosphere and in the crown area. Its application as a wheat-bran/peat preparation that served as a food base probably contributed to its successful establishment (23). In soil samples taken at the end of the first growing season from the crown area of *Trichoderma*-treated plants, population counts of the antagonist reached 740 cfu/g of soil. In the untreated plots, however, the natural soil population of *Trichoderma* spp. declined below 10 cfu/g of soil during the same period. The failure of natural soil populations of *Trichoderma* spp. to survive in soil around the crown area apparently reflects the lack of nutrients and energy sources confronting the soil microflora (3). On the other hand, introducing the antagonist to the root zone along with a suitable food base presumably promotes its proliferation in the rhizosphere.

The proliferation of *T. harzianum* in the crown region of the plant had no significant effect on the *Fusarium* population in the same soil samples taken from field plots during the growing season. Nevertheless, disease incidence was significantly reduced. Similar results were obtained after mixing a *Trichoderma* preparation with soil artificially infested with *F. oxysporum* f. sp. *vasinfectum* (22). This indicates that competition might be associated with this control. Indeed, Baker (4) has already mentioned that biological control resulting from competition has little or no effect on the viability of pathogens in soil.

Application of *T. harzianum* as a seed treatment effectively reduced the incidence of crown rot of tomatoes. The same antagonist applied by the same method controlled *Fusarium* wilt of cotton, melon, and wheat (22).

After seed treatment, under field conditions, *T. harzianum* was recovered from tomato root segments. The highest counts of the antagonist were found on segments including the higher part of the root and the root tips. Similarly, Weller (26) observed that a marked antibiotic-resistant strain of pseudomonad applied on wheat seeds was the dominant bacterium colonizing the root tips of plants emerged from treated seeds 1-2 mo after

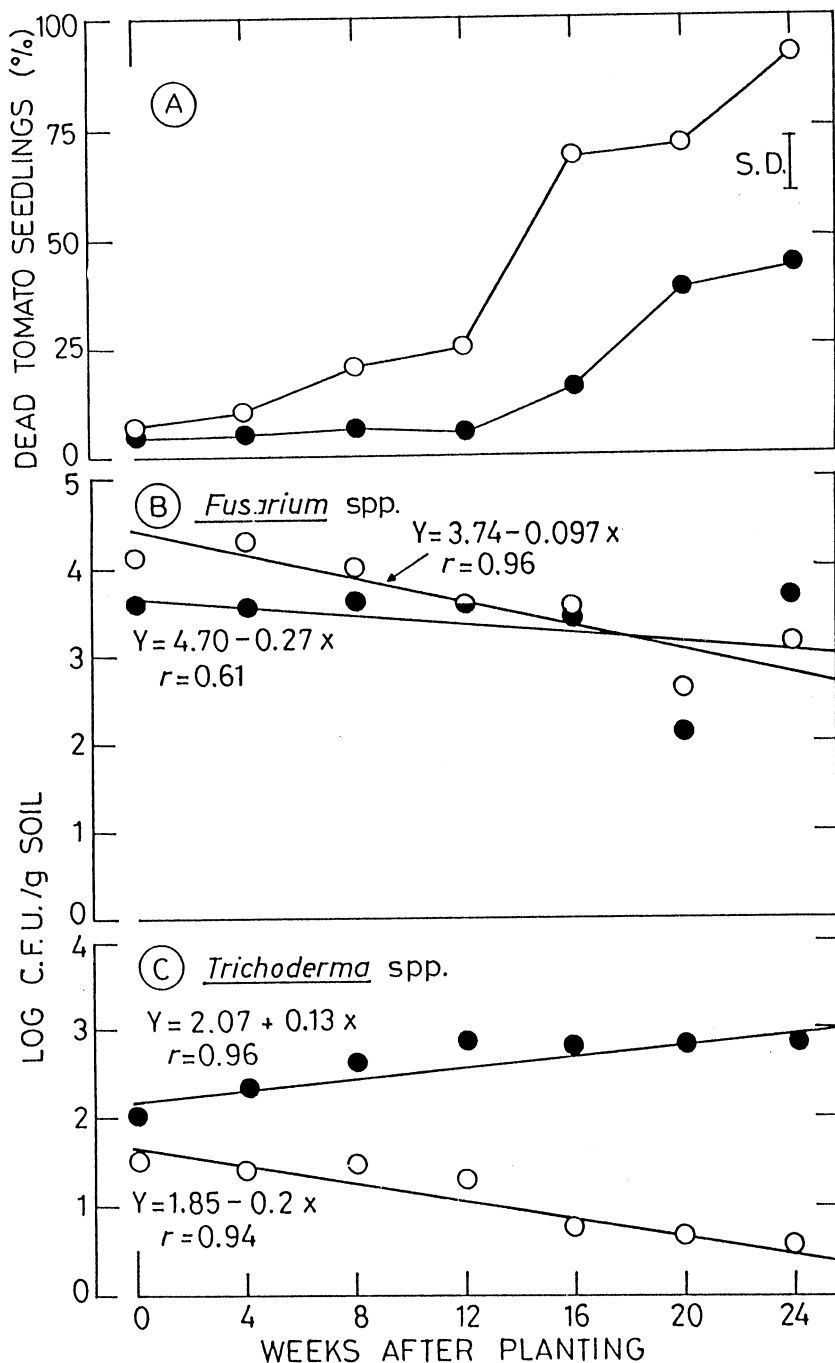


Fig. 3. (A) Inoculum potential of *Fusarium oxysporum* f. sp. *radicis lycopersici*. Population densities of (B) *Fusarium* spp. and of (C) *Trichoderma* spp. as affected by wheat bran/peat application of *T. harzianum* (●-●) compared with untreated control (o-o). Soil samples were taken during the growing season from plots of field experiment 1 at a distance of 5-10 cm from the plant crown. These samples were sown in the greenhouse with (A) untreated tomato seeds. Changes in population density levels of (B) *Fusarium* and (C) *Trichoderma* spp. in the soil samples were determined by linear regression analysis with a significance level of  $P = 0.05$ ;  $r$  values higher than 0.7 were significant.

planting. According to Cook and Baker (7), pathotypes of *F. oxysporum* invade the vascular system through the undifferentiated xylem in the juvenile portion of roots where the endodermis is not yet formed. Similarly, in the case of other important root pathogens, the juvenile root tip is the region in need of protection. In our study, counts of *Fusarium* spp. decreased mainly on root tips on which the highest counts of the antagonist were recorded. The effectiveness of *T. harzianum* in reduction of *F. oxysporum* root population indicates that the antagonist is adapted to serve as an effective competitor on the rhizoplane. Recently, Ahmad and Baker (1) reported that the rhizosphere competence of *Trichoderma* spp. was directly correlated with competitive saprophytic ability.

Discussing the colonization of plant

roots by microorganisms, Cook and Baker (7) indicated that any root-colonizing microorganism given the advantage of being the first to colonize the root, as may occur with seed treatment, has the potential to preempt the nutrient supply of pathogens.

The usefulness of biocontrol agents is increased by the fact that soil disinfestation does not control *Fusarium* crown rot on tomato as effectively as it controls other soilborne pathogens (19), and a complete soil drench with captafol is impractical when a plastic mulch is maintained during the entire growing season (15). Our study reveals that *T. harzianum* is effective in controlling *Fusarium* crown rot of tomato under field conditions; however, the possibility of integrating chemical and biological control should be investigated further.

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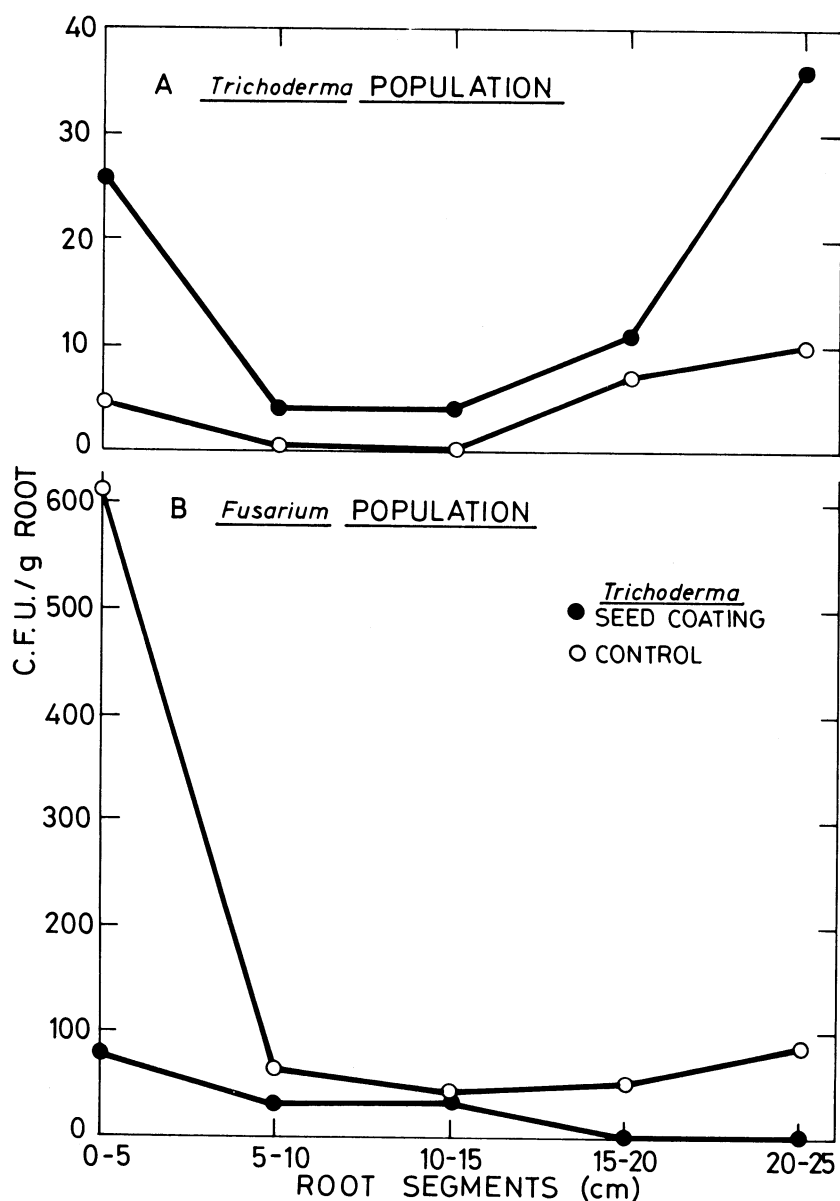


Fig. 4. Effects of seed coating with *Trichoderma harzianum* on colonization of tomato root segments by (A) *T. harzianum* and by (B) *Fusarium* spp. under field conditions. Untreated seeds served as controls.

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