

The Effect of the *Trichoderma harzianum* Strains on the Growth of Tomato Seedlings

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Abstract

An experiment was performed with commercial and noncommercial *Trichoderma harzianum* strains to test whether they have any effect on the growth of tomato seedlings. The tomato (*Lycopersicon esculentum* Mill. cv. 'Caruso') seedlings were grown in a greenhouse and watered daily by hand. 18-day old seedlings were inoculated with *Trichoderma harzianum* strains Plantshield™, T22, and T95 (10^7 conidia plus mycelial fragments/ml) and transplanted into plastic pots filled with Pro-Mix™ potting mix. Randomized complete block design was used and treatments were replicated three times. At six weeks, the seedlings were sampled for growth comparisons on seedling emergence, number of true leaves, fresh and dry weights of roots and shoots, stem caliper and shoot height. The data were subjected to ANOVA and the means tested by LSD. The results demonstrated that *Trichoderma harzianum* strains improved tomato seedling growth. There were differences between the untreated control and the treatments for all of the growth parameters at 4 weeks after inoculation with the exception of root fresh and dry weight.

INTRODUCTION

Tomato is one of the most important vegetable crops in the U.S.A. According to the agricultural statistics in 1997, total area in which tomato has been grown is 185,353 hectares. Total production of fresh and processing tomatoes is approximately 13 million tons and the estimated value of production is \$ 1.6 billion in 1996 (Anonymous, 1997). In tomato production, the U.S.A. ranks second in the world after China. Dollar value of the production could be much higher than the amount above if we can reduce the losses due to poor growing media, poor seedlings, plant diseases and the cost for chemicals to control diseases. Plant diseases, especially root diseases, cause significant losses in tomato production. For example, soil-borne plant pathogens cause seed rot, damping-off, root rot, wilt and fruit rot, which result in an annual \$4-5 billion in the United States alone (Jewell, 1987). To remain competitive with the leading countries in tomato production, growers in United States must increase yields and offset production costs.

Growing quality tomato transplants offers a number of benefits, in more economic production and convenience, to both commercial vegetable growers and home gardeners. To produce and market profitable crops, growers often depend on earliness, which can best be achieved by setting out well-grown and properly aged transplants. Transplant production in containers using potting media reduces plant mortality during field establishment and gives early and uniform crop yields (McKee, 1981). By using quality transplants, producers can insure a good stand of vegetable plants without the uncertainty of direct seeding (Courter et al., 1984). The fungus *Trichoderma harzianum* is a biological control organism against a wide range of soil-borne pathogens and has plant growth-promote capacity. It has been shown that *T. harzianum* stimulated the growth of tomato plants (Chet, 1990; McGovern et al, 1992; Datnoff and Pernezny, 1998). The purpose of current experiment was to determine the effects of *Trichoderma harzianum* strains on growth of tomato seedlings under greenhouse conditions

MATERIALS AND METHODS

An experiment was conducted in the greenhouse facilities at W.D. Holley Plant Environment Research Center, Colorado State University, Fort Collins, CO, U.S.A. to test the effect of three known *T. harzianum* strains on tomato transplant growth. Tomato (*Lycopersicon esculentum* Mill.) cv. 'Caruso' was used in the experiment. 'Caruso' is an older beefsteak cultivar of tomato still popular with greenhouse growers because of its good flavor. It matures to a rich red color with an average weight of 180-225 g. 'Caruso', which has sparse foliage, is best adapted to fall cropping and may produce yellow-shouldered fruit under high light intensities. The seeds were provided by Hydro-Gardens, Colorado Springs, CO, U.S.A.

Three strains of biocontrol fungi were evaluated in this experiment. *T. harzianum* strain T95 (T95) was provided by Suzanne M. Nemeth, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, Colo., U.S.A. *T. harzianum* strain KRL-AG2 (PlantShield™, 1×10^7 colony forming units/g as a wettable powder) was supplied by Bioworks Inc., Geneva, N.Y.). *T. harzianum* strain 1295-22 (T-22) was derived from 14-day old cultures grown on Potato Dextrose Agar (PDA) plates incubated at 25°C. PlantShield™ and T22 have the same active ingredient; only difference in this study was preparation of the strains for inoculum. T22 and T95 were maintained on PDA and kept at 4°C. PlantShield™ was maintained in the container provided by the manufacturing company and kept at 4°C. Fungal inoculum (10^7 conidia/ml) of strain T22 and T95 was prepared by blending 2 week-old PDA-grown cultures of the fungus with sterile distilled water, straining the suspension through cheesecloth. Conidial densities in the suspension were determined by use of a hemacytometer under a light microscope. PlantShield™ inoculum was applied according to company protocol (0.5-1.0g/L). Inoculation was performed by dipping the roots in the appropriate microbial suspension for 30 min.

Tomato seeds (cv. 'Caruso') were sterilized in a 1% bleach solution (NaOCl) for 30 min and rinsed thoroughly in sterile distilled water. The seeds were then soaked in 50 ml of a suspension (10^7 conidia/ml) of each of the *T. harzianum* strains (T95, T22 and PlantShield™) and incubated 30 min. Control seeds were soaked in an equal volume of distilled water. Treated and untreated control seeds were directly sown into plug trays filled with Pro-Mix™ BX peat-lite planting mix. Plug trays were placed on a bench in greenhouse. Seedling emergence was monitored for 14 days after seeding to determine biocontrol agent's effects on germination. 18 day-old tomato seedlings from each treatment were removed from plugs and potting mix was gently washed off of the root system. Transplant dip solution from each *T. harzianum* strain was prepared to a concentration of 10^7 conidia/ml. Bare tomato transplant roots were fully submerged in the solution for 30 minutes and immediately planted into 10 cm x 10 cm square plastic pots filled with Pro-Mix™ BX planting mix. An untreated control was included to the experiment. Untreated seedling roots were dipped in distilled water for 30 min. Five tomato seedlings were grown for each treatment/replication. The seedlings were watered by hand on daily basis and complete nutrient solution was applied with each watering. The nutrient solution consisted of CHEM-GRO tomato formula (Hydro-Gardens, Colorado Springs, CO, U.S.A), calcium nitrate and magnesium sulfate. CHEM-GRO tomato formula contains 4% N, 18% P₂O₅, 38% K₂O, 0.80 % Mg, 0.20, B, 0.05% Cu, 0.40% Fe, 0.40% Mn, 0.01% Mo, 0.05% Zn, and 2% Cl. The effects of *T. harzianum* strains on the growth of tomato seedlings were evaluated after 6 weeks from sowing. Five tomato seedlings from each treatment were removed from pots and planting mix was gently washed off of the root system. Number of leaves, shoots height, stem caliper at the soil line, shoot fresh weight, and shoot dry weight, root fresh weight, and root dry weight of tomato seedlings were recorded. Plant heights were measured from the soil line to shoot apices. Shoots and roots were dried 43°C for four days to obtain dry weight determinations (McGovern et al., 1992).

Root colonization by *T. harzianum* strains (T22, T95, and PlantShield™) was estimated in a separate experiment conducted in greenhouse again. Tomato seeds (cv.

‘Caruso’) sterilized in a 1% bleach solution (sodium hypochlorite) for 30 min and rinsed thoroughly in sterile distilled water were directly sown into 20 cm x 4 cm plastic tubes (designed especially for colonization studies) filled with Pro-Mix™ BX peat-lite planting mix inoculated with a spore suspension (10^7 conidia/ml) of each *T. harzianum* strains prepared as previously described. An untreated control was included to the experiment. Experiment was terminated when seedlings were 4 weeks old. Root systems were rinsed with tap water to remove potting mix particles. Root samples collected were cut into small fragments (1 cm long). Surface-disinfested root fragments were transferred onto acidic PDA (5 fragments/plate), and incubated at 25°C for 5 days. The percent *Trichoderma* root colonization was recorded from the number of roots yielding at least one colony of the target organism.

All tests were repeated once and included three replicates per treatment. The treatments were arranged in a randomized complete block design with five seedling plots with three replicates of each treatment. Data were analyzed by analysis of variance (ANOVA) and the means were separated by using Fisher’s LSD tests at alpha values of 0.05. Statistical analyses were conducted using the general linear models procedure of SAS Version 8e (SAS Institute Inc., Cary, NC, U.S.A.).

RESULTS AND DISCUSSION

The analysis of variance of the data showed significant differences in treatments at $P \leq 0.05$ level. PlantShield™ increased seedling emergence by 17% compared with control. However, *T. harzianum* T22 and T95 had no effect on emergence of tomato seedlings (Table 1). The biocontrol agent strains were not better or worse than each other in the effect on number of true leaves. All the strains increased true leaf number and shoot height of the seedlings compared with the control. PlantShield™ did not affect stem caliper while T22 and T95 increased stem caliper of tomato seedlings (Table 1). T22 and T95 increased shoot fresh and dry weights. None of the *T. harzianum* strains had an effect on root fresh and dry weights (Table 2).

There was no difference among the strains in colonizing roots of 4-week old tomato seedlings. Root colonization by *T. harzianum* strains Plantshield™, T22 and T95 was 93%, 100% and 100% respectively, and did not differ significantly (Table 3). Control plant roots had no colonization by any of the strains.

One of the most important characteristics necessary for acceptance and effectiveness of biocontrol agents is their ability to survive in the environments other than their origin and colonize plants roots during certain period of time to control plant pathogens (Nemec et al., 1996). In this study all three strains have maintained their populations at high levels after inoculation in the period of 4 weeks. This validates the other studies (Sivan and Chet, 1993; Nemec et al., 1996; Datnoff and Pernezny, 1998).

The concept of adding biocontrol agents into a planting mix or applying directly to the roots of transplants is an efficient, inexpensive means to provide a more vigorous transplant with disease protection when it is transplanted to the field (Nemec et al, 1996). In addition to their biocontrol activities, *Trichoderma* spp. have been reported to promote plant growth (Chang et al, 1986; Inbar et al., 1994). Possible explanation of this phenomenon includes control of minor pathogens leading to stronger growth and nutrient uptake (Ousley et al, 1993). In our study, the results indicated that *T. harzianum* strains had a positive effect on tomato transplant growth. However, further investigation is needed to confirm these results.

Literature Cited

- Anonymous, 1997. Agricultural Statistic, Annual USDA National Statistic Service.
Chang, Y.C., Baker, R., Kleifeld, O. and Chet, I. 1986. Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. Plant Disease 70:145-148.
Chet, I. 1990. Biological control of soil-borne plant pathogens with fungal antagonists in combination with soil treatments. p. 15-25. In: D. Hornby, (Ed.), Biological Control

- of Soilborne Plant pathogens. C.A.B. International, Wallingford, UK.
- Courter, J.W., Gerber, M., Vandemark, J.S. and Jacobsen, B.J. 1984. University of Illinois at Urbana-Champaign, Cooperative Extension Service, Circular 884.
- Datnoff, L.E. and Pernezny, K.L. 1998. Effect of bacterial and fungal microorganisms to colonize tomato roots, improve transplant growth and control of Fusarium Crown and Root Rot. 1998 Florida Tomato Institute Proceedings PRO 111:26-33.
- Inbar, J., Abramsky, M., Cohen, D. and Chet, I. 1994. Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. Eur. J. Plant Path. 100:337-346.
- Jewell, L.D. 1987. Agricultural Statistics. 1987 U.S. Department of Agriculture Research Service.
- McGovern, R.J., Datnoff, L.E. and Tripp, L. 1992. Effect of mixed infection and irrigation method on colonization of tomato roots by *Trichoderma harzianum* and *Glomus intraradix*. Proc. Fla. State Hort. Soc. 105:361-363.
- McKee, J.M.T. 1981. Physiological aspects of transplanting vegetables and other crops. I. Factors which influence reestablishment. *Horticultural Abstracts*, Farnham Royal, 51:265-272.
- Nemec, S., Datnoff, L. and Strandberg, J. 1996. Efficacy of biocontrol agents in planting mixes to colonize plant roots and control root diseases of vegetables and citrus. Crop Protection 15:735-742.
- Ousley, M.A., Lynch, J.M. and Whips, J.M. 1993. Effect of *Trichoderma* on plant growth; a balance between inhibition and growth promotion. Microb. Ecol. 26:277-285.
- Sivan, A. and Chet, I. 1993. Integrated control of Fusarium Crown and Root Rot of tomato with *Trichoderma harzianum* in combination with methyl bromide or soil sterilization. Crop Protection 12:380-386.

Tables

Table 1. Effects of biological treatments on seedling emergence, number of true leaves, stem caliper and shoot height of six-week old tomato transplants.

Treatment	Seedling Emergence (%)	# of True leaves	Stem caliper (mm)	Shoot height (cm)
Control	83.41 b*	3.61 b	5.37 b	27.83 c
Plantshield™	100.00 a	3.71 ab	5.16 b	30.27 b
<i>T. harzianum</i> T22	83.33 b	4.16 a	6.06 a	30.71 b
<i>T. harzianum</i> T95	88.89 b	3.89 ab	5.93 a	34.35 a

* Numbers in a column followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's LSD test.

Table 2. Effects of biological treatments on shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of six-week old tomato transplants.

Treatment	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Control	38.58 b*	4.09 bc	9.20 a	0.76 a
Plantshield™	37.83 b	3.57 c	8.68 a	0.61 b
<i>T. harzianum</i> T22	43.06 a	4.54 ab	10.28 a	0.78 a
<i>T. harzianum</i> T95	43.10 a	4.68 a	9.13 a	0.87 a

* Numbers in a column followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's LSD test.

Table 3. Percentage root colonization of tomato seedlings by *T. harzianum* strains.

Treatment	Colonization ¹ (%)
Control	0 b ²
Plantshield™	93 a
<i>T. harzianum</i> T22	100 a
<i>T. harzianum</i> T95	100 a

¹ The percent *Trichoderma* root colonization was recorded from the number of roots yielding at least one colony of the target organism (see Materials and Methods).

² Numbers in a column followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's LSD test.