http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (2): 253-257, 2004
ISSN 1028-8880

Effect of *Trichoderma harzianum* Strains to Colonize Tomato Roots and Improve Transplant Growth

[1]Nusret Ozbay and [2]Steven E. Newman
[1]Kahramanmaras Sutcu Imam University, Ziraat Fakultesi, Bahce Bitkileri Bolumu, Kahramanmaras-46100, Turkey
[2]Colorado State University, Department of Horticulture and Landscape Architecture, 105 Shepardson Building, Fort Collins, CO 80523-1173 USA

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 (10^7conidia plus mycelial fragments ml^{-1}) 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.

Key words: *Lycopersicon esculentum,* growing media, root colonization, Caruso, growth parameters

INTRODUCTION

Tomato is one of the most important vegetable crops in the U.S.A.[1] According to the agricultural statistics in 2002; the total area in which tomato has been grown is 180,000 hectares. Total production of fresh and processing tomatoes is approximately 13 million tons and the estimated value of production was $1.8 billion in 1996[2]. In tomato production, the USA ranks second in the world after China (Anonymous, 2002). 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[3]. 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 loss in the United States alone[3]. 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[4]. To produce and market profitable crops, growers often depend on earliness, which can 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[5]. By using quality transplants, producers can insure a good stand of vegetable plants without the uncertainty of direct seeding [4].

Adding biocontrol agents into a planting mix or applying directly to the roots of transplants is an efficient and inexpensive means to provide a more vigorous transplant with disease protection when it is transplanted to the field[6]. Many saprotrophic fungi, particularly certain isolates of *Trichoderma* species, can provide plant growth promotion in the absence of any major pathogens[7,8]. *Trichoderma* spp. are common inhabitants of the rhizosphere and are biological control organisms against a wide range of soilborne pathogens[9]. The application of *Trichoderma* strains to the soil as biological control agents, in the greenhouse and under field conditions, not only resulted in reduced disease incidence and severity but also enhanced plant growth[8,10,11-14]. Increased plant growth induced by

Corresponding Author: Dr. Nusret Ozbay, Kahramanmaras Sutcu Imam University, Ziraat Fakultesi, Bahce Bitkileri Bolumu, Kahramanmaras-46100, Turkey, E-mail: nozbay@ksu.edu.tr

Trichoderma spp. was demonstrated, in the absence of pathogens, in experiments conducted in autoclaved soil rooting medium[15,16]. The purpose of the current experiment was to determine the effects of *Trichoderma harzianum* strains on the growth of tomato seedlings under greenhouse conditions in soilless growing medium. This study was carried out to look at the effects of different strains and formulations of a biocontrol agent on tomato transplants grown in different media than the previous studies.

MATERIALS AND METHODS

An experiment was conducted at 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 two *T. harzianum* strains and two formulations on tomato transplant growth.

Plant material: Tomato *(Lycopersicon esculentum* Mill.) cultivar Caruso was used in the experiment. The seeds were provided by Hydro-Gardens, Colorado Springs, CO, U.S.A.

Preparation of fungal inoculum: Two strains of *T. harzianum* were evaluated in this experiment. *T. harzianum* strain T95 (T95) was kindly provided by Suzanne M. Nemeth, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO. *T. harzianum* strain KRL-AG2 (PlantShield™, $1x10^7$ colony forming units g^{-1} as a wettable powder) was supplied by Bioworks Inc., Geneva, N.Y. A second formulation of *T. harzianum* strain 1295-22 (T-22) was derived from 14-day old cultures (derived from strain KRL-AG2 grown on Potato Dextrose Agar (PDA) plates incubated at 25°C. Plantshield and T22 have the same active ingredient; the only difference in this study was the 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 (10^7microconidia + macroconidia ml^{-1}) 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 sterile 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 (0.5-1.0 $g^{-1}L^{-1}$). Inoculation was performed by dipping the roots in 20 ml of the appropriate microbial suspension for 30 min.

Plant growth conditions and treatments: Tomato seeds were sterilized in a 1% solution of bleach (containing 6% sodium hypochlorite) for 30 min and rinsed twice thoroughly in sterile distilled water. The seeds were then soaked in a 50 ml suspension (10^7 conidia ml^{-1}) of each *T. harzianum* strain (T95, T22 and PlantShield™) and incubated 30 min. Control seeds were soaked in an equal volume of distilled water. Treated and untreated control seeds (5/treatment/rep.) were directly sown into plug trays filled with Pro-Mix™ BX planting mix. Plug trays were placed on a bench in the greenhouse. The temperature was maintained at 18°C night and 25°C day. Seedling emergence was monitored for 14 days after seeding to determine the 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. A transplant dip solution from each *T. harzianum* strain was prepared to a concentration of 10^7conidia ml^{-1}. Bare tomato transplant roots were fully submerged in 20 ml of the solution for 30 min and immediately planted into 10 cm x 10 cm square plastic pots filled with Pro-Mix™ BX planting mix. Untreated seedling roots were dipped in distilled water for 30 minutes. Five tomato seedlings were grown for each treatment/replication. The seedlings were watered by hand on a 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 (15.5-0-0) and magnesium sulfate (9.9% Mg). CHEM-GRO™ tomato formula contains 4% N (total nitrogen), 18% P_2O_5, 38% K_2O, 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. The 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 at 43°C for four days to obtain dry weight determinations[17].

Root colonization by the *T. harzianum* strains: Root colonization by *T. harzianum* (T22, T95 and PlantShield™) was estimated in a separate experiment conducted in the greenhouse. Tomato seeds sterilized in a 1% bleach solution (containing 6% sodium hypochloride) for 30 min and rinsed thoroughly in sterile distilled water, were

directly sown into 20 cm x 4 cm plastic tubes filled with Pro-Mix™ BX planting mix inoculated by injecting a 20 ml spore suspension (10^7conidia ml^{-1}) of each *T. harzianum* strain prepared as previously described. An untreated control was included in the experiment. The experiment was terminated when seedlings were 4 weeks old. Root systems were rinsed with tap water for two min. to remove potting mix particles. Root samples were collected and cut into small fragments (1cm-long). Samples were surface-disinfested by immersion for 2 min. in a 3% bleach (containing 6% sodium hypochlorite) solution. Root fragments (5 fragments $plate^{-1}$) were transferred onto PDA acidifiedto pH: 4.5. and incubated at 25°C for 5 days in darkness. The percent *Trichoderma* root colonization was recorded from the number of roots yielding at least one colony of the target organism. On PDA, the perimeters of the colonies are white to cottony in appearance with green spores giving a pale to dark green color to the centre of the colony.

Experimental design and data analysis: 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

Root colonization: There were no significant differences among the strains or formulations in the levels of root colonization on 4-week old tomato seedlings. Root colonization of tomato seedlings by *T. harzianum* strains T22 and T95 was usually at 100% and 93% by Plantshield™ (Table 1). 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[6]. Colonization of the surface of the seeds or roots has frequently been seen to be a desirable trait for biocontrol activity [13,16] .In this study all three strains have maintained their populations at high levels after inoculation in the period of 4 weeks. This agrees with the prior studies[18,6,12].

Table 1: Percentage root colonization of tomato seedlings by *T. harzianum* strains

Treatment	Colonization[1] (%)
Control	0b[2]
PlantshieldTM	93a
T. harzianum T22	100a
T. harzianum T95	100a

[1] The percent *Trichoderma* root colonization was recorded from the number of fragments/roots yielding at least one colony of the target organism.
[2] Numbers in a column followed by the same letter are not significantly different (P =0.05) according to Fisher's LSD test

Table 2: 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	83.41b*	3.61b	5.37b	27.83c
Plantshield™	100.00a	3.71ab	5.16b	30.27b
T. harzianum T22	83.33b	4.16a	6.06a	30.71b
T. harzianum T95	88.89b	3.89ab	5.93a	34.35a

Table 3: 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	38.58b*	4.09bc	9.20a	0.76a
Plantshield™	37.83b	3.57c	8.68a	0.61b
T. harzianum T22	43.06a	4.54ab	10.28a	0.78a
T. harzianum T95	43.10a	4.68a	9.13a	0.87a

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

Transplant growth: The potential of *Trichoderma harzianum* strains to induce increased growth of tomato transplants was evaluated. The analysis of variance of data showed significant differences in treatment effects at $P \leq 0.05$. PlantShield™ significantly increased seedling emergence (17%) compared with control. However, *T. harzianum* T22 and T95 had no significant effect on emergence of tomato seedlings (Table 2). The biocontrol agent strains and formulations were not significantly different in the effect on number of true leaves (Table 2). All of the strains and formulations significantly increased shoot height (Table 2) of the seedlings compared with control and one increased the number of true leaves. PlantShield™ did not affect stem caliper while T22 and T95 significantly increased stem caliper of tomato seedlings (Table 2). T22 and T95 increased shoot fresh weight and T95 increased dry weight (Table 3). None of the *T. harzianum* strains or formulations had a significant effect on root fresh and dry weights (Table 3). Interestingly enough PlantShield™ gave lower root dry weight than the untreated control. One possible explanation for this result is that PlantShield™ might complete its shelf life by the time it was used in the experiments although it should be good according to the label. Most of these results are in agreement with earlier

studies while some of the results (root fresh and dry weights) are in disagreement with previous studies where treatments caused an increase in root and dry weights[15,19].

Trichoderma spp. have been reported to promote plant growth[8,10,20]. *T. harzianum* and *Paenibacillus macerans* alone or in combination significantly affected the growth of tomato transplants in the greenhouse and after outplanting into the field 30 days later. In the greenhouse, petiole numbers were increased between 6 to 9%, heights 8 to 18.8%, stem caliper 10 to 13.6%, leaf area 7 to 21%, petiole fresh weight 25 to 38% and root fresh weight 50%. In the field, petiole numbers were increased between 3 to 5%, heights 2 to 8% and stem caliper 1 to 7%[19]. In comparison our strains increased height between 11 to 26%, stem caliper 1 to 13%, number of true leaves 1 to 13%, shoot fresh weight 27%, root fresh weight 29%, shoot dry weight 9 to 14%.

Possible explanations of this phenomenon include; control of minor pathogens leading to stronger growth a nutrients uptake[11], solubilization of insoluble minor nutrients in soil[21] and production of growth hormones[15]. *Trichoderma* spp. may enhance plant growth by increasing the solubility of zinc, copper, iron and manganese ions, all plant nutrients with low solubility[20-22]. *T. harzianum* also increases plant nitrogen efficiency[22]. *T. harzianum* 1295–22 was shown to solubilize phosphate and micronutrients that could be made available toprovide plant growth[21]. Yedidia *et al.*[20] reported that an increase of 90% in phosphorus (P) and 30% in iron (Fe) concentration was observed in *T. harzianum* inoculated cucumber plants. They concluded that the improvement of plant nutritional level might be directly related to a general beneficial growth effect of the root system following *T. harzianum* inoculation. The results of present study is in the line of earlier studies indicated that *T. harzianum* strains had a positive effect on tomato transplant growth.

REFERENCES

1. Jones, B. Jr., 1999. Tomato plant culture in the field, greenhouse and home garden. CRS Press, New York. pp: 216.
2. Anonymous, 2002. Vegetables 2001 Summary. Agricultural Statistics Board. National Agricultural Statistics Service, U.S. Department of Agriculture. USA.
3. Jewell, L.D., 1987. Agricultural Statistics. U.S. Department of Agriculture Research Service. USA.
4. Courter, J.W., M. Gerber, J.S. Vandemark and B.J. Jacobsen, 1984. Growing vegetable transplants. University of Illinois at Urbana-Champaign, Cooperative Extension Service, Circular 884.
5. 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.
6. Nemec, S., L.E. Datnoff and J. Strandberg, 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.
7. Whipps, J.M., 1997. Developments in the biological control of soil-borne plant pathogens. Advances in Botanical Res., 26: 1-134.
8. Inbar, J., M. Abramsky, D. Cohen and I. Chet, 1994. Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. European J. Pl. Pathol., 100: 337–346.
9. Chet, I., 1987. *Trichoderma*-application, mode of action and potential as a biocontrol agent of soilborne plant pathogenic fungi. In: Innovative Approaches to Plant Disease Control. I. Chet (Ed.). John Wiley and Sons. New York, pp: 137-160.
10. Chang, Y.C., Y. Chang, R. Baker, O. Kleifeld and I. Chet, 1986. Increased growth of plant in the presence of the biological control agent *T. hazianum*. Plant Dis., 70: 145-148.
11. Ousley, M.A., J.M. Lynch and J.M. Whips, 1993. Effect of *Trichoderma* on plant growth; a balance between inhibition and growth promotion. Microbial. Ecology, 26: 277-285.
12. Datnoff, L.E., S. Nemec and K. Pernezny, 1995. Biological control of Fusarium crown and root rot of tomato in Florida using *Trichoderma harzianum* and *Glomus intraradices*. Biological Control, 5: 427-431.
13. Harman, G. E. and T. Bjorkman, 1998. Potential and existing uses of *Trichoderma* and *Gliocladium* for plant disease control and plant growth enhancement. In: '*Trichoderma* and *Gliocladium*' Volume 2. (Eds. G.E. Harman and C.P. Kubicek), Taylor and Francis, London, pp: 229-265.
14. Datnoff, L.E. and K.L. Pernezny, 1998. Effect of bacterial and fungal microorganisms to colonize tomato roots, improve transplant growth and control Fusarium crown and root rot. 1998 Florida Tomato Institute Proceedings PRO 111: 26-33.
15. Windham, M.T., Y. Elad and R. Baker, 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathol., 76: 518-521.
16. Kleifeld, O. and I. Chet, 1992. *Trichoderma harzianum*: Interaction with plants and effect on growth response. Plant and Soil, 144: 267-272.
17. McGovern, R.J., L.E. Datnoff and L. Tripp, 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.

18. Sivan, A. and I. Chet, 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.
19. Datnoff, L.E. and K.L. Pernezny, 2001. *Paenibacillus macerans* and *Trichoderma harzianum* enhance transplant growth and suppress fusarium crown and root rot in Florida tomato production. 2001 Caribbean Division Meeting Abstracts, June 11-15, 2001 - La Habana, Cuba. Publication no. P-2002-0025-CRA.
20. Yedidia, I., A.K. Srivastva, Y. Kapulnik and I. Chet, 2001. Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. Plant and Soil, 235: 235-242.
21. Altomare, C., W.A. Norvell, T. Björkman and G.E. Harman, 1999. Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295–22. Applied Environ. Microbiol., 65: 2926-2933.
22. BARD, 2000. *Trichoderma harzianum:* Pugnacious But Friendly Fungus. United States-Israel Binational Agricultural Research and Development Fund. January 2000.