Reprint ISSN 1923-7766 (Web Version)

International Journal of Experimental Agriculture

(Int. J. Expt. Agric.)

Volume: 10 **Issue: 2** **July 2020**

Int. J. Expt. Agric. 10(2): 1-6 (July 2020)

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ISSN-1923-7766 (Online)
Int. J. Expt. Agric. 10(2):1-6(July 2020)

EFFECT OF INDIGENOUS *TRICHODERMA* STRAINS ON GROWTH OF TOMATO SEEDLINGS

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Accepted for publication on 28 July 2020

ABSTRACT

Islam MM, Shahid SB, Akter A, Hossain MS, Bhuiyan MSU (2020) Effect of indigenous *Trichoderma* strains on growth of tomato seedlings. *Int. J. Expt. Agric.* 10(2), 1-6.

Trichoderma spp. are beneficial to agriculture which are mainly used as biological control agents against a wide range of plant pathogens and for their plant growth enhancement capacity. An experiment was carried out with three Bangladeshi native strains namely *T. harzianum* TR05, *T. virens* TR06 and *T. asperellum* TR08 to evaluate the effects on growth of tomato seedlings in green house conditions. Completely Randomized Design (CRD) was followed and each treatment was replicated three times. Tomato seeds and three week old seedlings were inoculated with the above mentioned *Trichoderma* strains and transplanted into tray filled with autoclaved commercial culture soil. At two weeks after sowing, seedling emergence was monitored to determine effectiveness of the *Trichoderma* strains on germination. T_1 (Application of TR05) recorded highest germination (96.3%) followed by T_3 (Application of TR08; 90.0%) and T_2 (Application of TR06; 82.0%). At six weeks, the seedlings were compared for number of leaves, shoot length, root length, stem caliper at soil line, fresh weight, dry weight and seedling vigour. The results demonstrated that *T. harzianum* TR05 strain significantly improved the growth of tomato seedlings among the strains applied. Colonization of roots at 5-week old tomato seedlings was found significant. T_1 showed highest colonization (98.7%) followed by T_3 (90.3%) and T_2 (81.3%). The correlation between all aforesaid characters of tomato seedlings was found significant and ($P<0.05$) positive. More than 90% correlation was observed between the aforementioned growth characters of tomato seedlings.

Key words: *tomato seedlings, thrichoderma, growth characters, root colonization*

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most popular and widely grown vegetable in the world including Bangladesh. The popularity of tomato is rising among consumers, not only because of its good taste, but also for its high levels of vitamin C, lycopene, and beta-carotene, which are anti-oxidants that promote good health. It is also important in terms of area, production, yield, and commercial use, placed sixth based on total annual world production (FAO 2014). The high demand for tomato makes it a high value crop, can generate much income for farmers. Nowadays, land area under tomato cultivation both in summer and winter season, has been expanded day by day due to increasing domestic demand (BBS 2017). The production could be higher if we can minimize losses due to poor seedlings, poor growing condition, plant diseases, and chemical cost for controlling diseases. Plant diseases, especially root diseases, cause significant losses in tomato production. As for example, soil-borne pathogens cause seed rot, root rot, wilt, damping-off, collar rot and fruit rot, resulting annual losses in Bangladesh. Various methods have been investigated for controlling such pathogens, including chemical controls, cultural practices, resistant variety, plant extracts, volatile compounds and biological control. Although chemical control is an important method for eradication of pathogens in severe attack, it is not economical in the long term because it causes environmental pollution, leaves harmful residues and can lead to development of resistant strains among the target organisms with repeated use (Naseby *et al*. 2000). Biological control is an alternative to control the pathogen at low cost and to restore soil fertility without disturbing other components of the environment. To be at par with the leading countries in tomato production, growers in Bangladesh must increase yields and offset production.

Quality seedlings offer a lot of benefits, like higher production and less disease. To produce profitable crop, tomato growers depend on earliness, which may be achieved by properly grown aged seedlings. Seedlings production in tray/containers using commercial culture soil reduces transplants mortality at field establishment and resulting uniform and early yields (McKee 1981). By using quality seedlings, grower can ensure a good production without doubt of direct seeding (Courter *et al*. 1984).

The plant growth-promoting and antagonistic fungi *Trichoderma* contain a large number of strains that are beneficial to agriculture mainly as biological control agents (BCAs). They are well known for their biocontrol ability against a wide range of plant pathogens (Harman *et al*. 2004) and for their plant growth enhancement (Hoyos-Carvajal *et al*. 2009) under greenhouse and field conditions (Papavizas 1985; Sivan and Chet, 1992). However, their efficacy largely depends on the physical, chemical and biological soil conditions. *Trichoderma* species colonize numerous plant roots, decompose plant residues and are involved in the biodegradation (Anand *et al*. 2006). It has been reported that *Trichoderma* spp. stimulated the growth of tomato plants (Chet 1990; McGovern *et al*. 1992; Datnoff and Pernezny, 1998). In our previous study, we obtained three indigenous *Trichoderma* isolates; *T. harzianum* TR05, *T. virens* TR06 and *T. asperellum* TR08, from different locations of Bangladesh, which have potentials as effective biocontrol agents under *in vitro* conditions (Islam *et al*. 2016). Therefore, the aim of present study was to determine the effects of *Trichoderma* strains on growth of tomato seedlings in greenhouse conditions.

MATERIALS AND METHODS

Trichoderma strains

Three strains (*T. harzianum* TR05, *T. virens* TR06 and *T. asperellum* TR08) identified and isolated from various regions of Bangladesh (Islam *et al*. 2016) and maintained at Department of Plant Pathology and Seed Science laboratory in Sylhet Agricultural University, Bangladesh were used in this experiment. The experiments were carried out during 2015.

Treatment combinations

The effectiveness of the three *Trichoderma* strains on tomato seedlings was examined in a greenhouse following seed treatments. Inoculation of the *Trichoderma* strains were conducted according to the following combinations: T_0 = Control, T_1 = Application of TR05, T_2 = Application of TR06 and T_3 = Application of TR08.

Germination

A12 day-old PDA-grown culture of each *Trichoderma* (TR05, TR06 and TR08) strains was blended with sterile deionized water and a 30-ml fungal suspension was prepared. Spore density in the suspension was 7×10^8 spore/ml, determined by a haemocytometer under a light microscope. Tomato seeds were sterilized in 1% sodium hypochlorite solution for 10 minutes and rinsed thoroughly in sterile distilled water. Inoculation with the *Trichoderma* strains was then performed by dipping seeds in the fungal suspension for 30 min. Control seeds were soaked in an equal volume of deionized water. The treated and control seeds were directly sown into trays (30 × 20 × 7.5 cm) filled with autoclaved commercial culture soil (0.8 kg/tray) at the rate of 30 seeds per tray. Trays were placed on a bench in a greenhouse. For 2 weeks after sowing, seedling emergence was monitored to determine effectiveness of the *Trichoderma* strains on germination.

Growth characters of tomato seedlings

21-day-old tomato seedlings from each treatment were removed from tray and culture soil was gently washed off of the root system. Seedlings from each *Trichoderma* strain was dipped into a solution at concentration of 7×10^8 spore/ml. Tomato seedling roots were submerged in the solution for 30 minutes and immediately transplanted into plastic pots (6″ diameter with 8″ height) filled with 1.3 kg of autoclaved commercial culture soil. Untreated seedling roots were dipped in distilled water for 30 minutes and included to the experiment. Five tomato seedlings were grown for each treatment. The seedlings were watered by hand on daily basis. The effects of *Trichoderma* strains on growth of tomato seedlings were evaluated after 6 weeks from sowing. Five tomato seedlings from each treatment were removed from pots and the roots gently washed using running tap water. Number of leaves, shoot length, root length, stem caliper at soil line, fresh weight, dry weight and seedlings vigour were evaluated. Shoot length were calculated from soil line to shoot top. Dry weight measured after drying for 5 days at 45°C (McGovern *et al*. 1992). Seedling vigour was calculated using the following formula:

Vigour index = (Root length + Shoot length) × Seed germination percentage

Root colonization

Root colonization by *Trichoderma* strains (TR05, TR06 and TR08) was calculated in a separate experiment conducted in greenhouse. Tomato seeds were sterilized in 1% sodium hypochlorite solution for 10 minutes and rinsed thoroughly in sterile distilled water, and directly sowninto trays (30 × 20 × 7.5 cm) filled with autoclaved commercial culture soil (0.8 kg/tray), inoculated with a spore suspension (7×10^8 spore/ml) of each *Trichoderma* (TR05, TR06 and TR08) strains as previously described. An untreated control was included in the experiment. Experiment was ended when seedlings were 5 weeks old. Seedlings from each treatment were removed from tray and root systems were rinsed with running tap water to remove soil particles. Roots were collected and cut into 3 cm long fragments, and then surface sterilized. Disinfested root fragments were transferred onto PDA (5 fragments/plate), and incubated at (25±2)°C for 7 days. The percent root colonization was calculated from the number of roots yielding at least one colony of the target organism.

Statistical analysis

The treatments were arranged in a Completely Randomized Design (CRD) with three replications of each treatment. The one-way analysis of variance (ANOVA), descriptive statistics and bivariate correlation of growth characters data was analyzed using statistical software SPSS version 17.0. The treatment multiple comparison was calculated using least significant difference (LSD) at 5% level of significance. The treatment mean values were compared and lettering was done using Duncan multiple rang test (DMRT) at 5% level of significance.

RESULTS

The effects of *Trichoderma* strains (TR05, TR06 and TR08) showed significant difference among treatments (T_1, T_2 and T_3) at $P \leq 0.001$ level of significance on germination, number of true leaves, stem caliper and shoot length of tomato seedlings (Table 1). All the strains increased germination, true leaf number, stem caliper and shoot length of the seedlings compared with the control. T_1 showed highest germination percentage (96.3%) followed by T_3 (90.0%) and T_2 (82.0%). The highest number of true leaves recorded in T_1 (8.33) followed by T_3 (6.33). However, T_2 and T_0 had no effect on true leaves of seedlings. Similarly, maximum shoot length was

observed in T_1 (14.0 cm) followed by T_3 (10.3 cm), and T_2 and T_0 had no effect on shoot length of tomato seedlings. In case of stem caliper, T_1 (8.67 mm) increased stem caliper of tomato seedlings followed by T_3 (6.00 mm) and T_2 (4.33 mm).

Table 1. Effects of *Trichoderma* strains on germination, number of true leaves, stem caliper and shoot length of tomato seedlings

Treatments	Germination (%)	True Leaves	Stem Caliper (mm)	Shoot Length (cm)
T_0 (Control)	75.3±0.58d	3.67±0.58c	3.33±0.58d	6.67±1.53c
T_1 (Application of TR05)	96.3±0.58a	8.33±0.58a	8.67±0.58a	14.0±0.00a
T_2 (Application of TR06)	82.0±1.73c	4.33±0.58c	4.33±0.58c	7.00±0.00c
T_3 (Application of TR08)	90.0±2.00b	6.33±0.58b	6.00±0.00b	10.3±0.58b
Mean	85.92	5.67	5.58	9.50
SD	8.38	1.97	2.15	3.18
Min	75.00	3.00	3.00	5.00
Max	97.00	9.00	9.00	14.00
$LSD_{0.05}$	2.61	1.09	0.94	1.54
CV (%)	9.75	34.74	38.53	33.47
Level of significance	**	**	**	**

** indicates significant at P<0.001

Effects of *Trichoderma* strains also showed significant differences among treatments at P≤0.001 level of significance on root length, seedling vigour, fresh weight and dry weight of tomato seedlings (Table 2). All the strains showed expansion of root length, seedling vigour, fresh weight and dry weight of tomato seedlings over control. The highest root length was found in T_1 (12.3 cm) followed by T_3 (9.00 cm) and T_2 (6.67 cm), and that of lowest was observed in control (T_0; 4.50 cm). In case of seedling vigour, maximum vigour seedlings was observed in T_1 (2537.00) followed by T_3 (1740.67) and T_2 (1120.33), and that of minimum was in found control (T_0; 841.83). The highest fresh weight was recorded in T_1 (14.3 g) followed by T_3 (11.3 g) and T_2 (8.67 g), and the lowest fresh weight was found in control (T_0; 7.00 g). Similarly, maximum dry weight was obtained from T_1 (6.67 g) followed by T_3 (4.03 g) and T_2 (2.07 g), and that of minimum was found in control (T_0; 0.93 g).

Table 2. Effects of *Trichoderma* strains on root length, seedling vigour, fresh weight and dry weight of tomato seedlings

Treatments	Root Length (cm)	Seedlings Vigour	Fresh Weight (g)	Dry Weight (g)
T_0 (Control)	4.50±0.50d	841.83±157.52d	7.00±0.00d	0.93±0.06d
T_1 (Application of TR05)	12.3±1.15a	2537.00±119.41a	14.3±0.58a	6.67±0.58a
T_2 (Application of TR06)	6.67±0.58c	1120.33±41.50c	8.67±0.58c	2.07±0.12c
T_3 (Application of TR08)	9.00±0.00b	1740.67±88.10b	11.3±0.58b	4.03±0.06b
Mean	8.13	1559.96	10.33	3.43
SD	3.09	686.61	2.93	2.29
Min	4.00	675.00	7.00	0.90
Max	13.00	2619.00	15.00	7.00
$LSD_{0.05}$	1.30	207.42	0.94	0.56
CV (%)	38.01	44.01	28.36	66.76
Level of significance	**	**	**	**

** indicates significant at P<0.001

Colonization of roots of 5-week-old tomato seedlings was found significant (Table 3). Control seedlings roots had no colonization by any of the strains. T_1 showed highest colonization (98.7%) followed by T_3 (90.3%) and T_2 (81.3%).

Table 3. Effects of *Trichoderma* strains on root colonization of tomato seedlings

Treatments	Colonization (%)
T_0 (Control)	0.00±0.00d
T_1 (Application of TR05)	98.7±1.15a
T_2 (Application of TR06)	81.3±1.53c
T_3 (Application of TR08)	90.3±0.58b
Mean	67.58
SD	41.26
Min	0.00
Max	100.00
$LSD_{0.05}$	1.88
CV (%)	61.05
Level of significance	**

** indicates significant at P<0.001

The correlation coefficients between the studied characters are shown in Table 4. The correlation coefficient between all studied characters of tomato seedlings was found significantly (P<0.05) positive. High correlation (more than 90%) was observed between traits (*viz*, germination percentage, number of true leaves, stem caliper, shoot length, root length, seedlings vigor, fresh weight and dry weight of seedlings), while 66–85% correlation was observed between growth characters and colonization on the root of tomato seedlings.

Table 4. Correlation coefficients between different growth characters and colonization on the root of tomato seedlings

Characters	TL	SC	SL	RL	SV	FW	DW	CL
GP	0.945**	0.956**	0.934**	0.964**	0.968**	0.966**	0.970**	0.851**
TL		0.930**	0.959**	0.919**	0.955**	0.965**	0.947**	0.724**
SC			0.964**	0.986**	0.990**	0.960**	0.984**	0.736**
SL				0.947**	0.985**	0.946**	0.958**	0.658*
RL					0.986**	0.963**	0.987**	0.800**
SV						0.971**	0.991**	0.740**
FW							0.970**	0.787**
DW								0.763**

Note: GP= Germination percentage (%), TL= Number of true leaves, SC= Stem caliper (mm), SL= Shoot length (cm), RL= Root length (cm), SV= Seedlings vigour, FW= Fresh weight (g), DW= Dry weight (g) and CL= Colonization (%); **indicates correlation is significant at the 0.01 level (2-tailed) and *indicates correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

Application of three *Trichoderma* strains; *T. harzianum* TR05, *T. virens* TR06 and *T. asperellum* TR08 showed a significant impact on germination, number of true leaves, stem caliper, shoot length, root length, seedling vigour, fresh weight and dry weight of tomato seedlings compared to control (Table 1 and Table 2). *Trichoderma* strains could employ some mechanisms (both direct and indirect) which influence seed germination and seedling vigor (Zheng and Shetty, 2000; Clear and Valic, 2005). Rate of seed germination, rapid root growth and elongation during seed germination, number of leaves, plant height, stem caliper, fresh weight and dry weight of seedlings are important criterion of seedling vigor. In this study, our findings indicated that the effects of *Trichoderma* on seedling growth and vigor mainly depend on type of *Trichoderma* species/isolate/strain applied (Azarmi *et al*. 2011). This finding is agreed with the findings of Hajieghrari 2010; Ousley *et al*. 1994; Barker 1988. Some researchers also have reported that *Trichoderma* increased plant growth and productivity (Harman 2006; Manju and Mall, 2008). In some cases, *Trichoderma* have a stimulatory effect on plant growth by modifying the soil conditions. In addition, increased growth response of seedlings, caused by *Trichoderma* strains, mainly depend on the ability to survive and establishment in the rhizosphere (Harman 2006; Harman *et al*. 2004). Among the three *Trichoderma* strains, TR05 (*T. harzianum*) exhibited significant results of the above mentioned agronomic traits of tomato seedlings after inoculation. These studies have been confirmed in the case of *T. harzianum* enhancing seed germination, root and shoot length (Dubey *et al*. 2007) as well as increasing the frequency of healthy plants. Ozbay and Newman (2004) also reported that *T. harzianum* strains have significantly (P<0.05) increased the germination, stem caliper, height, shoot and root dry weight in tomato seedlings, transplanted into pots in green house. In case of root colonization by *Trichoderma* strains, TR05 showed maximum colonization (98.7%) than other strains (Table 3). This may be because TR05 was better able to colonize the rhizosphere of tomato seedlings than other strains (Bennett and Whipps, 2008). On the other hand, higher root colonization by *Trichoderma* was observed because of its high reproduction capacity (Woo *et al*. 2005). One of the most salient features of biocontrol agents is ability to survive in environments than their other origin and colonize numerous plants roots during certain period of time (Anand *et al*. 2006; Datnoff and Pernezny, 1998; Nemec *et al*. 1996). Root colonization by *Trichoderma* may be not only the root exudates such as amino acid and carbohydrates, but also by several factors that influence *Trichoderma*-seedling interaction. In this context, some *Trichoderma* strains may interconnect better with the seedlings in the same conditions (Harman 2006; Harman *et al*. 2004). The correlate on between agronomic traits indicates association of them and indirect effect on the growth of tomato seedlings. The current investigation revealed highly positive correlation between growth characters as well as between growth characters and root colonization of tomato seedlings, which are in partially agreement with the findings of Mahapatra *et al*. (2013) and Haydar *et al*. (2007). Therefore, it is assumed that any of the studied agro-morphologic traits (*viz*. germination percentage, number of true leaves, stem caliper, shoots length, root length, seedlings vigor, fresh weight as well as dry weight of seedlings) could be selected for tomato production. This is expected that development of root system and production of several organic acids (such as gluconic, citric and/or fumaric acids) in rhizosphere by *Trichoderma* that decrease soil pH, lead to increased solubility of insoluble compound, availability of micronutrient, as well as increase uptake of plant nutrient. Enhancement of plant nutrient uptake and its transportation from root to vegetative parts, along with produced plant stimulators lead to increase above mentioned agronomic traits of tomato seedlings proportionally (Azarmi *et al*. 2011).

CONCLUSION

Application of *Trichoderma* strains into soil or directly apply to roots of seedlings is an economic and effective way to obtain a more vigorous tomato seedlings, and transplanted to main field. In addition to providing antimicrobial action, *Trichoderma* can stimulate the biological activity of resident antagonistic microbial populations, thus promoting plant growth. Probable explanation of this circumstance includes control of certain pathogens resulting vigorous growth and nutrient uptake of seedlings/transplants. In this study, results indicated that *Trichoderma* strains had a positive impact on growth of tomato seedlings. However, further studies are underway in net house and field conditions to examine the effectiveness of these strains as alternatives to chemicals.

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