



## ***Trichoderma*-mediated enhancement of nutrient uptake and reduction in incidence of *Rhizoctonia solani* in tomato**

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### **Abstract**

*Trichoderma harzianum* is a naturally occurring filamentous fungus which solubilizes mineral nutrients and inorganic fertilizers, increasing availability and uptake of nutrients to the plant. *Rhizoctonia solani* is a major problem for seedlings, causing damping-off and in mature plants causing foot and root rot in the tomato crop, reducing nutrient uptake. The aim of this study is to evaluate the effect of *Trichoderma harzianum* (BHU-51), *Trichoderma harzianum* (BHU-105) and their consortium *Trichoderma harzianum* (BHU-51+BHU-105) on management of *R. solani* and nutrient levels in the plants.

The application of *Trichoderma* as a seed treatment significantly decreased the incidence of damping-off and increased the vigour index of the plants. The maximum reduction in disease incidence was recorded for the consortium (BHU-51+BHU-105) treatments. The mineral content in treated plants was also higher than untreated pathogen-inoculated controls. Field trials also showed that the consortium produced better results in terms of shoot length, chlorophyll content and yield than the control.

The application of *Trichoderma* in consortium form increased mineral nutrient uptake, reduced disease incidence and obtained a greater yield with reduced chemical pesticide loads, benefitting farmers and consumers.

**Keywords:** *Trichoderma*, *Rhizoctonia solani*, nutrient uptake, damping-off, disease management.

### **Introduction**

Natural agricultural soil harbours lots of agriculturally important beneficial microorganisms, such as *Trichoderma*, *Pseudomonas*, *Bacillus* spp., arbuscular mycorrhizal fungi, etc., which are responsible for sustaining plant health and the fertility of the soil. They affect plant growth either by reducing the deleterious effects of phytopathogenic organisms, increasing nutrient uptake, nutrient cycling, the synthesis and release of growth-promoting hormones (increasing plant vigour) or by preventing pathogen attack and thus acting as biocontrol agents (Jeffries et al. 2003, Glic 1995). The high and indiscriminate use of agrochemical inputs reduces the useful role and populations of rhizosphere microorganisms (Mader et al. 2002), since their efficiency depends mainly upon the physical and chemical properties of the soil. Among beneficial microorganisms, the filamentous fungus *Trichoderma* is one of the most important and widely used in agriculture as a biocontrol agent against many plant pathogens, and it may also increase plant growth (Shoresh et al. 2010), enhance the vigour of poor-quality seeds (Mastouri et al. 2010; Shoresh et al. 2010), improve the nitrogen-use efficiency of plants (Shoresh et al. 2010, Harman 2011) and solubilize micronutrients (Altomare et al. 1999). Chemical communication is established between the plant and *Trichoderma* when the most efficient strains infect and colonize the outer epidermal layers of the roots. Beneficial effects can occur for at least the first growing season: because the fungus grows and continues to colonize the roots, in turn the plant also shows increased growth. By this colonization and chemical communication, the physiology of the plant is strongly affected via changes in plant

gene expression (Samolski *et al.* 2012; Bae *et al.* 2011; Shores *et al.* 2010). Thus, the fungus reprogrammes plant gene expression, resulting in an alteration of plant responses to their environment.

Tomato is one of most important vegetable crops in India, cultivated round the year with an acreage of 3.5 million ha producing 10.3 million tons. *Rhizoctonia solani* causes seedling damping-off and foot/root rot in the mature plants, and is found consistently in the field. Control of this sclerotial pathogen is difficult because of its ecology; it has an extremely broad host range and a high survival rate of sclerotia under diverse environmental conditions. Efficient strategies of control are therefore needed. Chemical control causes many negative consequences including resistance, resurgence and residues which cause indirect effects on human health by accumulating in food or reducing food nutritional quality. Numerous studies have shown that biological control offers an environmentally friendly alternative for the protection of plants from soil-borne pathogens.

*Trichoderma* species are worldwide in occurrence and are present in nearly all soils and diverse habitats (Harman *et al.* 2004). These versatile fungi act on pathogens via various mechanisms such as mycoparasitism, antibiosis, competition, and nutrient competition by secreting antifungal metabolites. *Trichoderma* spp. also solubilize various plant nutrients such as Fe, Cu, Mn and Zn in soils, and even some natural fertilizers such as rock phosphate and pyrite (Altomare *et al.* 1999): this ability enhances plant growth and productivity. For improving crop yield and growth, and other beneficial effects of rhizosphere activity, the use of *Trichoderma* appears to be a promising alternative to pesticides and chemical fertilizers. In the present study we used a consortium of two different compatible *Trichoderma* isolates for increasing their overall efficiency. Specifically we tested the application of *Trichoderma harzianum* BHU51 (GenBank accession no. JN 618343.), *Trichoderma harzianum* BHU105 (GenBank accession no. JN 618344) and their consortium (BHU51+BHU105) on tomato seeds and seedlings, measuring nutrient uptake, plant growth and biocontrol under greenhouse and field conditions.

## Materials & Methods

The experiments were conducted under greenhouse and field conditions at the experimental farm of Banaras Hindu University, Varanasi, India. The soil type of the experimental field was sandy loam (Udico Ustochrept) with the following physicochemical and electrochemical properties: bulk density 1.41 Mg m<sup>-3</sup>, particle density 2.47 Mg m<sup>-3</sup>, water-holding capacity 44.38%, sand 512.6 g kg<sup>-1</sup>, silt 284.8 g kg<sup>-1</sup>, clay 202.6 g kg<sup>-1</sup>, pH<sub>w</sub> (1 : 2.5 in distilled water) 7.3, electrical conductivity 0.454 dSm<sup>-1</sup>, free CaCO<sub>3</sub> 0.32%, organic carbon 4.2 g kg<sup>-1</sup>, cation exchange capacity 11.8 {Cmol (p+) kg<sup>-1</sup>}, available nitrogen 252 kg ha<sup>-1</sup>, available phosphorus 21.3 kg ha<sup>-1</sup>, available potassium 158.3 kg ha<sup>-1</sup>.

*Trichoderma* isolates were isolated from different regions of Uttar Pradesh (India), characterized for their antagonistic and plant growth-promotion activity, and sequenced (data not shown). The two most efficient and compatible *Trichoderma* isolates were *Trichoderma harzianum* BHU51 (GenBank accession no. JN 618343) and *Trichoderma harzianum* BHU105 (GenBank accession no. JN 618344): they and their consortium (BHU51+ BHU105) were used in this study. The pathogen *Rhizoctonia solani* was isolated from infected tomato plants from the vegetable farm of Banaras Hindu University. The pathogen inoculum was prepared in sand maize meal media (Blestos *et al.* 1997).

Experiments were performed with four treatments and three replicates each. The plot size was 2×2 m<sup>2</sup> and the seedling distance was kept at 60 × 60 cm. The analysis used Anova implemented by SPSS-16. Four treatments combinations were arranged as in Table 1.

**Table 1:** Treatment combinations

Treatments	Description
T1	<i>Rhizoctonia solani</i>
T2	<i>Trichoderma harzianum</i> (BHU-51) + <i>T. harzianum</i> (BHU-105) + <i>R. solani</i>
T3	<i>T. harzianum</i> (BHU-51) + <i>R. solani</i>
T4	<i>T. harzianum</i> (BHU-105) + <i>R. solani</i>

Seeds of ‘Navodya’ tomato (*Lycopersicum esculentum* Mill.) were obtained from a local market. Seedlings were raised in pots filled with sterilized soil (121 °C and 15 lbs pressure for 1 h for two consecutive days) and transplanted into the field after 30 days of sowing.

*Trichoderma* formulations containing approximately  $10^8$  spores/g were used for treating seeds and seedlings. For the combined inoculation (consortium), formulations were prepared separately and mixed in equal amount (1:1) before treatment. A slurry of the formulations was made and then seeds added to the slurry, mixed and allowed to soak for 30 min. The treated seeds were placed in sterile Petri dishes and air-dried on a laminar flow bench overnight at room temperature. Control seeds were treated with mixtures of CMC suspension and talcum powder only. Thirty-day-old seedlings were used for field trials. Seedlings were treated by being dipped into the *Trichoderma* suspensions prepared as above and left for half an hour before transplanting. Surface-disinfected seeds were first inoculated with mycelial suspension of pathogens followed by talc preparations of *Trichoderma* isolates separately. The pathogen was grown in liquid culture medium for a week and homogenized before being used as an inoculum. *Trichoderma* isolates were tested for their plant growth promotion activity using the standard roll-towel method (ISTA 1993). The germination percentage of seeds was recorded and the vigour index calculated as described by Baki & Anderson (1973).

Under glasshouse and field conditions we tested the ability of *Trichoderma* isolates to reduce damping-off of seedlings, induce and increase the emergence of seedlings, increase plant height, and fresh and dry weight. Trials of the two *Trichoderma* isolates and their consortium were carried out in pots kept under glasshouse conditions. Pots were filled with sterilized soil and mixed with pathogen inoculates at a rate of 5g per kg of soil. The pots containing inoculum were incubated for seven days at room temperature. Treated dry tomato seeds were planted into pots according to treatment. One hundred seeds were planted per pot and evaluated for percent germination and damping-off of seedlings. Seedling emergence was counted at regular intervals. The germination and cumulative damping-off of seedlings were recorded 30 days after sowing. The incidence of damping off in seedlings was expressed as a percentage of the total number of plants. The number of seedlings which had survived, and the shoot length, root length and fresh and dry weight of shoot and root of surviving plants were recorded from each replicate. To measure fresh and dry weights, plants were washed under running tap water to remove all soil from the roots and then dried at 80 °C in a drying oven after recording their fresh weight. After 72 hr, plant dry weights were recorded. For the field study, inoculates of *R. solani* comprising 100g per m<sup>2</sup> (grown on sand maize media) were inoculated in the field seven days before transplanting.

Plant samples for study of minerals in seedlings were taken 30 days after sowing. The plant material was dried at 65 °C for 48 hr. The dried samples were separately ground, and passed through a 0.5 mm sieve for analysis of mineral components. The micro-Kjeldahl procedure (IITA 1982) used for analysis of total Nitrogen. Total phosphorus content was determined by the Vanadomolybdate method using a spectrophotometer; flame photometry was carried out for the analysis of K and Ca, while Zn, Mn, Mg, Cu and Fe were determined by atomic absorption spectrophotometry.

In the field, transplanted seedlings were observed at regular intervals for the symptoms of wilting, foot rot or damping off, and for signs of *R. solani*. Disease severity was estimated by

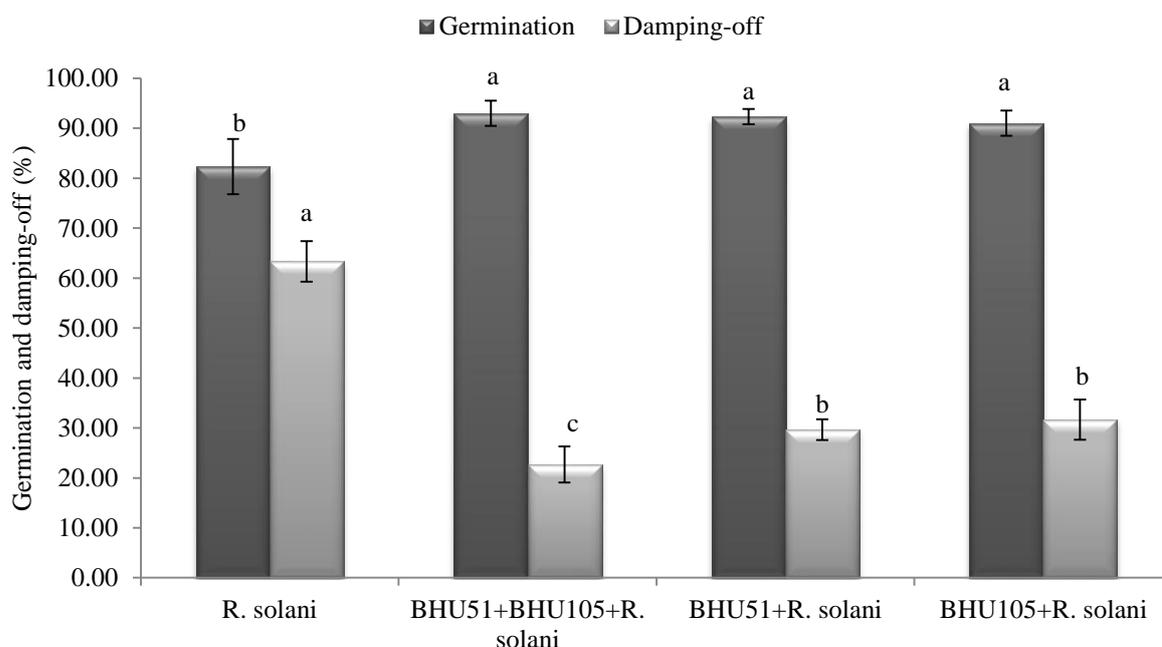
scoring individual plants. The severity of stem lesions was scored on the 0-5 scale described by Sneh *et al.* (1966) and Ferry & Dukes (2002). The mean disease rating and Percent disease reduction were calculated by the formula given by Pal *et al.* (2001).

The measurement of shoot length was carried out at 60 days after transplanting. The yield was measured at regular intervals, and the cumulative yield was expressed in kg/plot. The analysis of chlorophyll content was done by harvesting fresh leaves at 60 days after transplanting and weighed to determine fresh weight. The leaves were crushed in 80% acetone, centrifuged, and the amount of chlorophyll determined by taking the absorbance at 645 and 663 nm using a spectrophotometer (Arnon 1949). The result was expressed as mg chlorophyll per gram fresh weight.

## Results

### Plant growth promotion activity

Seeds treated with *Trichoderma* BHU-51, BHU-105 and their consortium (BHU-51+BHU-105) significantly increased seed germination compared to the pathogen-inoculated control (Fig. 1), with the consortium the highest value. The incidence of damping-off was maximal in the untreated control, and lowest in seeds treated with the consortium (Fig 1).



**Fig. 1:** Effects of *Trichoderma harzianum* (BHU-51), *Trichoderma harzianum* (BHU-105) and their consortium (BHU-51+ BHU-105) on the germination and incidence of damping-off of tomato caused by *Rhizoctonia solani*. Bars with different letters indicate statistically significant differences among treatments using Duncan's multiple range test ( $p < 0.05$ ).

Treated seeds had significantly increased shoot and root lengths compared to control plants, and treatment also had beneficial effects on fresh and dry shoot and root weights (Table 2). The vigour index was maximal in the consortium treatment and lowest in the untreated pathogen-inoculated seeds (Table 2).

**Table 2:** Efficacy of *Trichoderma* isolates on growth attributes and incidence of tomato inoculated by *Rhizoctonia solani*

Treatments	Shoot length (cm)	Shoot fresh weight (mg)	Shoot dry weight (mg)	Root length (cm)	Root fresh weight (mg)	Root dry weight (mg)	Vigor index
Control ( <i>R. solani</i> )	9.44 ± 0.38c	290.67 ± 12.58c	19.47 ± 1.00c	2.05 ± 0.19c	11.93 ± 1.80c	2.33 ± 0.32c	345.74 ± 58.72d
BHU -051+BHU-105+ <i>R. solani</i>	12.83 ± 0.47a	583.27 ± 7.13a	44.40 ± 3.14a	3.18 ± 0.39a	33.83 ± 2.08a	4.02 ± 0.25a	1393.17 ± 55.20a
BHU- 051+ <i>R. solani</i>	11.56 ± 0.10b	562.03 ± 10.75ab	37.63 ± 2.65b	2.62 ± 0.11b	25.70 ± 1.77b	3.04 ± 0.21b	1246.50 ± 57.47b
BHU-105+ <i>R. solani</i>	11.44 ± 0.21 b	543.33 ± 15.50b	36.67 ± 4.51b	2.60 ± 0.17b	24.03 ± 1.86b	2.94 ± 0.36b	1127.08 ± 51.40c
LSD (p= 0.05)	0.72	25.62	4.46	0.45	4.33	0.56	120.04

All the values represent the mean of three replicates ± standard deviation. Different letters denote a statistically significant difference according to Duncan's Multiple Range Test; we give also the Least Significant Difference (LSD) at p=0.05.

**Table 3:** Effect of *Trichoderma* and *Rhizoctonia solani* on nutrient uptake in tomato seedlings, measured 30 days after sowing

Treatments	N (%)	P (%)	K (%)	Mg (%)	Ca (%)
Control ( <i>R. solani</i> )	1.31 ± 0.13c	0.13 ± 0.01c	1.61 ± 0.08c	0.40 ± 0.02d	1.19 ± 0.09c
BHU -051+BHU-105+ <i>R. solani</i>	2.27 ± 0.12a	0.28 ± 0.02a	2.14 ± 0.09a	0.76 ± 0.05a	1.75 ± 0.07a
BHU- 051+ <i>R. solani</i>	1.76 ± 0.19b	0.22 ± 0.01b	1.93 ± 0.07b	0.67 ± 0.04b	1.47 ± 0.08b
BHU-105+ <i>R. solani</i>	1.99 ± 0.24ab	0.20 ± 0.02b	1.86 ± 0.08b	0.51 ± 0.04c	1.35 ± 0.04b
LSD (P= 0.05)	0.26	0.03	0.11	0.08	0.11

as Table 1

**Table 4:** Effect of *Trichoderma* and *Rhizoctonia solani* on micro-nutrient uptake in tomato seedlings

Treatments	Zn (ppm)	Mn (ppm)	Cu (ppm)	Fe(ppm)
Control ( <i>R. solani</i> )	26.10 ± 2.10c	29.00 ± 1.73d	5.83 ± 0.87b	76.13 ± 4.90c
BHU -051+BHU-105+ <i>R. solani</i>	36.93 ± 1.97a	42.87 ± 1.40a	10.37 ± 0.91a	100.47 ± 3.97a
BHU- 051+ <i>R. solani</i>	31.33 ± 1.63b	39.00 ± 1.37b	9.07 ± 0.45a	91.20 ± 6.68ab
BHU-105+ <i>R. solani</i>	30.33 ± 1.80b	32.60 ± 1.35c	6.00 ± 0.44b	87.73 ± 3.59b
LSD (P= 0.05)	4.23	3.27	1.42	9.03

as Table 1

The mineral content of treated seedlings showed significantly higher N, P, K, Ca and Mg levels in comparison with the untreated pathogen-inoculated control (Table 3). Seed treated with the consortium exhibited maximal N, P, K, Mg and Ca content. There was no significant difference in N content between BHU-51+BHU-105 and BHU-105 treatments, whereas significantly higher contents of P, K, Ca, and Mg were usually recorded in consortium versus either or both singly treated seeds (Table 3). The micronutrient contents of Zn, Mn, Cu and Fe were also recorded as significantly higher in the *Trichoderma*-treated plants in comparison with the controls (Table 4). Again the maximum contents of Zn, Mn, Cu and Fe were recorded in the consortium treatment, followed by single *Trichoderma*-treated treatments, with the lowest contents in the untreated pathogen-inoculated control.

**Table 5:** Effect of *Trichoderma* isolates on chlorophyll content, disease incidence and yield of tomato against *Rhizoctonia solani*

Treatments	Shoot length (cm)	Chlorophyll content, (mg g <sup>-1</sup> fw)	Mean disease rating	Disease reduction (%)	Yield (kg/plot)
<b>2008-09</b>					
Control ( <i>R. solani</i> )	55.0 ± 3.6c	0.75 ± 0.05b	3.13 ± 0.15a	-	6.6 ± 0.5c
BHU -051+BHU-105+ <i>R. solani</i>	79.7 ± 2.5a	1.07 ± 0.01a	1.57 ± 0.12c	45.1 ± 0.9a	11.9 ± 0.9a
BHU- 051+ <i>R. solani</i>	70.0 ± 3.6b	0.99 ± 0.05a	1.88 ± 0.07b	39.1 ± 2.0b	9.6 ± 0.5b
BHU-105+ <i>R. solani</i>	73.0 ± 3.6ab	0.99 ± 0.04a	1.85 ± 0.09b	39.9 ± 1.9b	9.1 ± 0.5b
LSD (P= 0.05)	6.7	0.08	0.20	3.2	1.3
<b>2009-10</b>					
Control ( <i>R. solani</i> )	50.7 ± 3.2c	0.79 ± 0.10c	3.42 ± 0.17a	-	6.9 ± 0.5c
BHU -051+BHU-105+ <i>R. solani</i>	76.0 ± 2.0a	1.02 ± 0.07a	1.58 ± 0.07c	47.1 ± 2.7a	12.3 ± 0.9a
BHU- 051+ <i>R. solani</i>	69.7 ± 2.1b	0.99 ± 0.03a	2.00 ± 0.09b	40.1 ± 1.4b	9.2 ± 0.7b
BHU-105+ <i>R. solani</i>	66.7 ± 3.1b	0.99 ± 0.04a	1.88 ± 0.14b	41.8 ± 2.2b	8.3 ± 1.0b
LSD (P= 0.05)	5.7	0.14	0.26	4.2	1.6

As Table 1

In the field trials, seedlings treated with the consortium exhibited maximal shoot lengths, significantly longer than singly treated seedlings, which were significantly longer than the untreated pathogen-inoculated controls (Table 5), a pattern repeated in both trials. Chlorophyll content also showed the same pattern (Table 5). Mean disease rating (Table 5) was highest in the untreated pathogen-inoculated control and lowest in the consortium treatment, with intermediate values for the singly treated plants, all significantly different from one another. The disease reduction was significantly higher in the consortium than single treatments, again repeated across years (Table 5). The beneficial effect of *Trichoderma* was also observed in the yield parameter, which were higher in the treatments than in the control (Table 5). Again the yield was significantly greater in the consortium treatment than singly treated plants, which were significantly greater than the controls.

## Discussion

The use of *Trichoderma* species as biocontrol agents of different soil-borne plant pathogenic fungi is well documented, as is their influence on seed germination and seedling vigour (Clear & Valic, 2005). The growth promotion activity in plants is usually determined by their shoot length, fresh weight and dry weight (Chang *et al.*, 1986, Kleifeld & Chet, 1992, Azarmi *et al.*, 2011), and *Trichoderma* spp. are known to do this (Samolski *et al.* 2012, Harman *et al.* 2012, Hermosa *et al.* 2012).

In this study two *Trichoderma* isolates were applied individually and together as a consortium in talc-based formulations for seed and seedling treatments. Our results indicated that the consortium of compatible isolates enhanced the growth and vigour of the plants compared with single isolates. Srivastava *et al.* (2010) also reported that the use of combinations of bioagents are more effective than when used singly, and also that biopriming of seeds with *Trichoderma* and fluorescent *Pseudomonas* increased seed germination and reduced the incidence of disease. Pandey & Maheshwari (2006) and Abeysinghe (2009) also reported that use of consortia of beneficial microbes can lead greater plant protection and growth promotion activity. Our results are similar to those of other authors (Hajieghrari, 2010; Ousley *et al.*, 1994) and may be due to the ability of *Trichoderma* isolates to survive and colonize in the root and rhizosphere (Harman, 2006; Harman *et al.* 2004). Root colonization by *Trichoderma* is affected by many factors, such as root exudates, soil conditions, and the species or isolate involved, and their source populations: all these factors influence the *Trichoderma* – plant interaction. Many researchers report that rhizosphere-competent isolates produce a number of metabolites in the rhizosphere which influence *Trichoderma* colonization and plant growth promotion (Vinale *et al.* 2008a, b). Under glasshouse conditions, it has been reported that without the addition of fertilizers, *Trichoderma* significantly increased the phosphorus and potassium content in the leaves of tomato (Inbar *et al.* 1994). In our experiments the plants were also not fertilized, and we also found significantly increased levels of nitrogen, phosphorus and potassium, especially in the consortium treatment. We conclude that the growth response and development of plants is probably caused by compounds of *Trichoderma* and increased uptake of mineral nutrients by treated seeds and seedlings.

Solubilization of mineral nutrients of soil by beneficial microbes enhances soil fertility. The production of organic acids such as gluconic, citric and fumaric acid by fungi such as *Trichoderma* sequester cations and acidify the microenvironment in the rhizosphere and decrease soil pH, which leads to increased solubility of mineral nutrients and plant nutrient uptake (Azarmi *et al.*, 2011, Cunningham & Kuiack, 1992), and ultimately photosynthetic rate and plant vigour. Increased concentrations of Ca, Mg, P, Na and K in the shoot and root following the application of *Trichoderma harzianum* T447 to the soil were reported by Azarmi *et al.* (2011). In our study not only macronutrients but also micronutrients were increased following *Trichoderma* treatment. Nzanza *et al.* (2011) also reported that the application of *Trichoderma* increased nitrogen; the combination of *Trichoderma* and mycorrhiza increased Mn and Zn content, whereas mycorrhiza alone increased the uptake of P and S in tomato plants. Samolski *et al.* (2012) reported that the *qid-74* gene induced modification in root architecture that increased total absorptive surface area in response to increased nutrient uptake, ultimately increasing plant biomass. It has also been reported that it is possible to reduce nitrogen application rates by 30–50% with no reduction in yield by the use of *Trichoderma* (Harman 2011; Shores *et al.* 2010) in several crops. Kaya *et al.* (2009) also reported that observed increases in plant growth might be due to increased solubility of relatively insoluble plant nutrients caused by *Trichoderma* species. Altomare *et al.* (1999) and Yedidia *et al.* (2001) reported that *T. harzianum* has the ability to solubilize plant nutrients (rock phosphate, MnO<sub>2</sub>, Fe<sub>2</sub>O<sub>3</sub>, Cu and metallic Zinc) from their solid phase. *T. harzianum* T-22 reduced oxidized metallic ions which increased their solubility, and also produced siderophores that chelated iron (Altomare *et al.* 1999).

In this study the increase in chlorophyll content of the leaves validated the advantage of *Trichoderma*-treated seedlings over the controls, increasing photosynthetic efficiency (Vargas *et al.* 2009). Our data on the vigor index and yield showed that the consortium was far better than the single treatments. Treated seedlings were more resistant to damping-off caused by *R. solani*, again maximal in the consortium treatment. The relative contributions of *Trichoderma* spp. to the suppression of *Rhizoctonia* damping-off have been discussed by many researchers

(Kwok *et al.*, 1987; Lumsden & Locke, 1989; Lewis & Papavizas, 1985) in species such as cotton, sugarbeet and radish seedlings in the greenhouse.

*Trichoderma* was clearly beneficial in biocontrol, with the mean disease rating greatest in the untreated control and lowest in the consortium treatment, mirrored in the data for the maximum percent disease reduction. The second field trial used the same fields and hence there were successive crops from the same soil: there were therefore increased inocula of soil-borne pathogens in the control plots, which thereby increased the mean disease rating in the growing season. Elad *et al.* (1980) reported that incorporation of *Trichoderma harzianum* in pathogen-infested soil significantly reduced bean disease caused by *R.solani* and *Sclerotium rolfsii*. Similar results for disease reduction were recorded by Pal *et al.* (2001) using plant growth promoting rhizobacteria against *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graminearum*. The ability of *Trichoderma* species to reduce the incidence of fungal diseases is well known, and is mainly due to mycoparasitism, antibiosis and competition for nutrients in the rhizosphere (Harman & Lumsden, 1990; Sivan & Chet, 1986, 1992). *Trichoderma* penetrate the root cortex, increasing lignification and inducing resistance in treated plants against pathogen attack (Kleifeld & Chet, 1992). *Trichoderma* also increase activities of the hydrolytic enzyme chitinase and  $\beta$ -1, 3-glucanase, and also stimulate the plant defense mechanism (Shoresh *et al.* 2010, Lorito *et al.* 2010, Singh *et al.* 2011, Druzhinina *et al.* 2011). Being an endophytic plant symbiont, *Trichoderma* induces gene expression in plants, which probably changes the physiology of plant in such a way as to improve resistance to abiotic and biotic factors, increase uptake of nitrogen fertilizer and photosynthetic efficiency and ultimately increase plant growth and productivity (Harman 2012, Hermosa *et al.* 2012).

We conclude that the application of beneficial microorganisms such as *Trichoderma* in combinations of strains (as in the consortium here) are more useful than as individual strains because two or more compatible isolates of same or different species work synergistically to give enhanced impact. The production of vigorous seedlings with more resistance to soil-borne plant pathogenic fungi is advantageous to the producers as well as to the farmers. Increased uptake of mineral nutrients reduces the quantity of inorganic fertilizer used, and reductions in the incidence of diseases reduce the burden of chemical pesticides on the crop, both of which benefit farmers and consumers alike.

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### References

- Abeyasinghe S (2009) Efficacy of combined use of biocontrol agents on control of *Sclerotium rolfsii* and *Rhizoctonia solani* of *Capsicum annuum*. *Archiv für Phytopathologie und Pflanzenschutz* 42(3): 221–227
- Altomare C, Novell WA, Bjorkman T & Harman GE (1999) Solubilization of phosphate and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. *Applied Environmental Microbiology* 65: 2926-2933
- Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* 24: 1-15
- Azarmi R, Hajieghrari B & Giglou A (2011) Effect of *Trichoderma* isolates on tomato seedling growth response and nutrient uptake. *African Journal of Biotechnology* 10(31): 5850-5855
- Bae H, Roberts DP, Lim HS, Strem M, Park SC, Ryu CM *et al.* (2011) Endophytic *Trichoderma* isolates from tropical environments delay disease onset and induce resistance against *Phytophthora capsici* in hot pepper using multiple mechanisms. *Molecular Plant Microbe Interactions* 24: 336–351
- Baki AAA & Anderson JD (1973) Vigour determination in soybean seed by multiple criteria. *Crop Science* 13: 630-633
- Chang YC, Chang YC, Baker R, Kleifeld O & Chet I (1986) Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. *Plant Disease* 70: 145-148

- Celar F & Valic N (2005) Effects of *Trichoderma* spp and *Gliocladium roseum* culture filtrates on seed germination of vegetables and maize. *Journal of Plant Diseases & Protection* 112(4): 343-350
- Cunningham JE & Kuiuack C (1992) Production of citric and oxalic acids and solubilization of calcium phosphate by *Penicillium bilaii*. *Applied Environmental Microbiology* 58: 1451-1458
- Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E, Mukherjee PK, Zeilinger S, Grigoriev IV & CP Kubicek (2011) *Trichoderma*: the genomics of opportunistic success, *Nature Reviews Microbiology* 9:749-759
- Elad Y, Chet I & Katan J (1980). *Trichoderma harzianum*: a biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. *Journal of Phytopathology* 70: 119-121
- Fery RL & Dukes SrPD (2002) Southern blight (*Sclerotium rolfsii* Sacc.) of cowpea: yield-loss estimates and sources of resistance. *Crop Protection* 21: 403–408
- Glick BR (1995) The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* 41: 107–120
- Hajieghrari B (2010) Effects of some Iranian *Trichoderma* isolates on maize seed germination and seedling vigor. *African Journal of Biotechnology* 9(28): 4342-4347
- Harman GE (2006) Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology* 96(2): 190-194
- Harman GE, Herrera-Estrella AH, Horwitz BA & Lorito M (2012). Special issue: *Trichoderma* - from basic biology to biotechnology. *Microbiology* 158: 1-2
- Harman GE, Howell CR, Viterbo A Chet I & Lorito M (2004) *Trichoderma* species - opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology* 2: 43-56
- Harman GE (2011) Multifunctional fungal plant symbionts: new tools to enhance plant growth and productivity. *New Phytologist* 189: 647–649
- Hermosa R, Viterbo A, Chet I & Monte E (2012) Plant-beneficial effects of *Trichoderma* and of its genes. *Microbiology* 158: 17–25
- IITA (1982) Automated and semi-automated methods of soil and plant analysis manual, series No.7. IITA, Ibadan, Nigeria
- Inbar J, Abramsky M, Cohen D & Chet I (1994) Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. *European Journal of Plant Pathology* 100: 337-346
- ISTA (1993) Proceedings of International Seed Test Association, International rule for seed testing. *Seed Science Technology* 21: 1-152
- Jeffries P, Gianinazzi S, Perotto S, Turnau K & Barea JM (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant and soil fertility. *Biology & Fertility of Soils* 37: 1–16
- Kaya C, Ashraf M, Sonmez O, Aydemir S, Tuna AL & Cullu MA (2009) The influence of arbuscular mycorrhizal colonization on key growth parameters and fruit yield of pepper plants grown at high salinity. *Scientia Horticulturae* 121: 1-6
- Kleifeld O & Chet I (1992) *Trichoderma*-plant interaction and its effect on increased growth response. *Plant & Soil* 144: 267-272
- Kwok OCH, Fahy PC, Hoitink HAJ & Kuter GA (1987) Interaction between bacteria and *Trichoderma hamatum* in suppression of *Rhizoctonia* damping-off in bark compost media. *Phytopathology* 77(80): 1206-1212
- Lewis JA & Papavizas GC (1985) Effect of mycelial preparation of *Trichoderma* and *Gliocladium* on populations of *Rhizoctonia solani* and the incidence of damping-off. *Phytopathology* 75(7): 812-817
- Lorito M, Woo SL, Harman GE & Monte E (2010) Translational research on *Trichoderma*: from ‘omics to the field. *Annual Review of Phytopathology* 48:395-417
- Lumsden RD & Locke JC (1989) Biological control of damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* with *Gliocladium virens* in soil-less mix. *Phytopathology* 79(3): 361-366
- Mader P, Fliebach A, Dubois D, Gunst L, Fried P & Niggli U (2002) Soil fertility and biodiversity in organic farming. *Science* 31:1694–1697
- Mastouri F, Bjorkman T & Harman GE (2010) Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. *Phytopathology* 100: 1213-1221
- Nzanza B, Marais D & Soundy P (2011) Tomato (*Solanum lycopersicum* L.) seedling growth and development as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi. *African Journal of Microbiology Research* 5(4): 425-431
- Ousley MA, Lynch JM & Whipps JM (1994) Potential of *Trichoderma* spp. as consistent plant growth stimulators. *Biology & Fertility of Soils* 17: 85-90
- Pal KK, Tilak KVBR, Saxena AK, Dey R & Singh CS (2001) Suppression of maize root diseases caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graminearum* by plant growth promoting rhizobacteria. *Microbiological Research* 156: 209-223
- Pandey P & Maheshwari DK (2006) Two-species microbial consortium for growth promotion of *Cajanus cajan*. *Current Science* 92: 1137–1141

- Samolski I, Rincon AM, Pinzon LM, Viterbo A & Monte E (2012) The *qid74* gene from *Trichoderma harzianum* has a role in root architecture and plant biofertilization. *Microbiology* 158: 129-138
- Shoresh M, Mastouri F & Harman GE (2010) Induced systemic resistance and plant responses to fungal biocontrol agents. *Annual Review of Phytopathology* 48:21-43
- Singh BN, Singh A, Singh SP & Singh HB (2011) *Trichoderma harzianum* mediated reprogramming of oxidative stress response in root apoplast of sunflower enhances defence against *Rhizoctonia solani*. *European Journal of Plant Pathology* 131: 121-134
- Sivan A & Chet I (1986) Possible mechanism for biological control of *Fusarium* spp. by *Trichoderma harzianum*. *British Crop Protection Conference. Pests and Diseases* 2: 865-872
- Sivan A & Chet I (1992) Microbial control of plant diseases. In: Mitchell R (ed), *New Concept in Environmental Microbiology*, Wiley, New York. pp 335-354
- Sneh B, Katan J, Henis Y & Wahl I (1966) Method for evaluating inoculum density of *Rhizoctonia* in naturally infested soil. *Phytopathology* 56: 74-78
- Srivastava R, Khalid A, Singh US & Sharma AK (2010) Evaluation of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt. *Biological Control* 53: 24-31
- Vargas WA, Mandawe JC & Kenerley CM (2009) Plant-derived sucrose is a key element in the symbiotic association between *Trichoderma virens* and maize plants. *Plant Physiology* 151: 792-808
- Vinale F, Sivasithamparam K, Ghisalberti E L, Marra R, Barbetti M J, Li H, Woo SL & Lorito M (2008a). A novel role for *Trichoderma* secondary metabolites in the interactions with plants. *Physiological & Molecular Plant Pathology* 72: 80-86
- Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Barbetti MJ Li H, Woo SL & Lorito M (2008b) *Trichoderma*-plant-pathogen interactions. *Soil Biology & Biochemistry* 40: 1-10
- Yedidia I, Srivastava AK, Kapulnik Y & Chet I (2001) Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. *Plant & Soil* 235: 235-242

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