

Non-rhizospheric *Trichoderma*- A Boon for Improving Nutrient uptake, Plant Growth and Yield in Tomato

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ABSTRACT: Fungal biocontrol agent, *Trichoderma* sp. found in many ecosystems, is known to grow as plant symbiont and promote plant growth and nutrition apart from biocontrol activity. We conducted a pot culture experiment to evaluate the efficacy of native *Trichoderma* isolates obtained from different locations and non-rhizospheric sources, previously identified effective in dual culture technique, in promoting nutrient use efficiency (NUE) in plants. Different application methods viz., seed treatment, soil application and their combination was analysed with untreated check and commercial isolate was used as standard check. The combination treatment was significantly superior in soil nutrient release and uptake by plants compared to control, along with plant growth and yield. Among different isolates, SMV, PSV and GMV were identified as most effective, which significantly increased the uptake of N, P, K, Ca, Mg and S compared to commercial isolate. Studying the sole contribution of native non-rhizospheric isolates of *Trichoderma* was a challenging job and by our experiment it was clear that the native isolates of *Trichoderma* obtained from non-rhizosphere sources exhibit not only significant antagonistic activity but also improved plant NUE thereby increasing plant growth and yield. This can be an important contribution to organic farming, natural farming and sustainable agriculture.

Keywords: Native isolates, nutrient uptake, plant growth, tomato, *Trichoderma* spp.

INTRODUCTION

Natural agricultural soil harbors 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 not only improve the plant growth by reducing the deleterious effects of phyto-pathogenic organisms, also by increasing nutrient uptake, nutrient cycling, synthesis and release of growth-promoting hormones and thus acting as biocontrol agents (Jeffries *et al.*, 2003; Glick, 1995).

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 (Vikas *et al.*, 2020), and it is also reported by many scientists all over the world to 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; Inbar *et al.*, 1994) and solubilize micronutrients (Altomare *et al.*,

1999).

Trichoderma spp. is a fungus which is extensively found in many regions of the world. These fungi are ubiquitous in a wide variety of environments, showing up in soil, forests, wood, FYM, saw dust, compost, straw, paper and other locations and sources apart from plant rhizosphere (Khoshmanzar *et al.*, 2020; Harman *et al.*, 2004). *Trichoderma* from non-rhizosphere sources have less competence with respect to microbial load and can be easily isolated. These have superior growth capability and are more efficient antagonists than those isolated from rhizosphere (Singh *et al.*, 2020; Harman *et al.*, 2004).

Soil being the most important substrate for plant growth has many nutrients in it existing in a sparingly soluble or insoluble state, which affects the circulation of nutrients in the soil to some extent. Nutrient use efficiency in crop is yield per unit of nutrient supplied from the soil and/or fertilizer (Sayaji and Prasun 2015). The nutrients that most commonly limit plant growth are N, P, K, S, and micronutrients. Nutrient availability to the plant is influenced by microorganisms present in the soil, soil edaphic factors and the rhizosphere.

Microbe-mediated improvement of nutrient use efficiency is gaining a lot of importance in the context of gradual loss of soil fertility/ productivity that has resulted from intensive agriculture (Sayaji and Prasun 2015).

Trichoderma species promote nutrient uptake by secreting organic acids to dissolve minerals and activate nutrients in the soil, leading to the circulation and utilization of nutrients in the soil (Bais *et al.*, 2006; Adams *et al.*, 2007; Barari, 2016). At the same time, due to the strong colonization ability of *Trichoderma* species, they expand the contact area between the rhizosphere and soil and increase the secretion of extracellular enzymes such as sucrase, urease, and phosphatase, as well as organic acids in the rhizosphere to improve nutrient cycling and enzyme activity in the soil (Inbar *et al.*, 1994; Harman, 2011).

Tomato (*Solanum lycopersicum* L.) is one of the important vegetable crops with high nutritional value, which is grown in almost all parts of India. In India, tomato was cultivated in 789.2 lakh ha and produced to a tune of 19.33 million tonnes with a productivity of 32.8 tons ha⁻¹ (Agristat, 2022). Among many diseases attacking tomato, *Fusarium* wilt (*Fusarium oxysporum* f. sp. *lycopersici*) is one of the important one, causes severe economic yield loss (Barari, 2016). In this context we have conducted an experiment to screen effective *Trichoderma* isolates obtained from different non-rhizospheric sources and identified as effective antagonists in dual culture technique in previous study by Harman *et al.* (2004). Our study will help to understand the influence of native *Trichoderma* isolates which are applied as different treatment modes, on nutrient release in soil and uptake by tomato plants which in turn improve the plant growth and yield.

MATERIALS AND METHOD

Five native, non-rhizospheric *Trichoderma* isolates identified effective in dual culture technique by Chaithra *et al.* (2020) and one commercial *Trichoderma* formulation were tested for their influence on NUE in tomato crop under green-house conditions using pot culture studies during summer season of 2020 at College of Agriculture, V. C. Farm, Mandya. Tomato cultivar Abhinav was used in the study. Five effective native *Trichoderma* isolates and different treatment combinations used in the study is mentioned in the Table 1 and 2, respectively.

A. Mass multiplication and formulation of *Trichoderma*
Trichoderma was mass multiplied on PDB media and formulated with sterilized talc powder. Initially sterile PDB media was inoculated with 7mm mycelial discs from 7 days old cultures of different *Trichoderma* isolates (from margin of active mycelial growth) and incubated at 28±2 °C for a week with intermittent stirring. To avoid bacterial contamination, PDB was added with 0.2g of streptomycin sulphate. After one week with cfu of 1×10⁸, mycelial mat was disintegrated by continuous shaking and later was mixed with sterile talc powder at 1:2 ratio (*Trichoderma*: talc) and dried under shade inside the laboratory under sterilized condition. After complete drying the formulated

product was packed in air tight polythene covers for future use. Three kilograms of talc formulation was prepared for each isolate for pot culture studies.

B. Quality test of *Trichoderma* formulation

The colony forming unit of (cfu/ml/g) broth culture and talc formulation of the isolates of *Trichoderma* spp. was enumerated using serial dilution method. The broth culture was mixed vigorously to disintegrate mycelial mat of *Trichoderma* into fine liquid form. Then, 10ml of culture was mixed with 100ml of sterile distilled water. In case of talc formulation, 10g of talc mixture of *Trichoderma* was mixed with 100ml of sterile distilled water. Then 1ml of this dilution (in both case) was transferred into sterile water blanks in the test tube containing 9ml sterile distilled water (10⁻¹) and from this dilution, 1ml was transferred to another water blank which gives rise to 10⁻² dilution. Same procedure was followed until 10⁻⁵ dilution. Then, 1ml from 10⁻⁵ dilution was transferred onto solidified PDA plates and was spread well all over the plate. Two plates were maintained for the dilution and incubated at 28°C for 3-4 days. Each day colony growth was observed and CFU of 1×10⁸ was maintained in formulation of all isolates for future work.

(i) Soil analysis. Soil samples were collected from potting mixture 7 days before the application of *Trichoderma* treatments and also 30 DAT and were analysed in the laboratory for available N, P, K, exchangeable Ca, Mg, and available S (Table 3).

Available nitrogen. Five gram of soil was distilled with 25 ml of 0.32 per cent potassium permanganate (KMnO₄) and 25 ml of 2.5 per cent NaOH. The ammonia released was trapped in 4 per cent boric acid containing mixed indicator and titrated against standard sulphuric acid (Subbiah and Asija 1956).

Available phosphorus

Available phosphorus content of soil was extracted using Olsen's extractant and the concentration of phosphorus in the extract was determined by chromolybdic blue colour method using spectrophotometer at 660 nm (Jackson, 1973).

Available potassium. Available potassium was extracted from soil using neutral N ammonium acetate at 1:5 soil to extractant ratio and the concentration of potassium in the extract was determined by flame photometer (Jackson, 1973).

Exchangeable calcium and magnesium.

Exchangeable Ca and Mg were extracted from soils using neutral N ammonium acetate at 1:5 soils to extractant ratio. The concentrations of Ca and Mg in the extract were determined by EDTA titration method as outlined by Jackson (1973).

(ii) Plant analysis. Tomato plant sample was collected 30 days after transplanting for nutrient analysis. Above ground plant sample was collected and fresh weight was measured. Then the sample was dried in hot air oven at 70±2°C for 48 hours, was ground to fine powder and was used for further analysis. Different methods used for plant analysis is given in Table 4.

Total nitrogen. Total nitrogen content of plant samples was determined by Kjeldahl's digestion distillation method. Powdered sample of 0.5 g was digested using

concentrated H₂SO₄ in presence of digestion mixture (containing K₂SO₄ and CuSO₄. 5H₂O and selenium in the ratio 100: 20: 1) and distilled in alkaline medium. The liberated NH₃⁺ was trapped in 4% boric acid containing mixed indicator and titrated against standard H₂SO₄ as described by Piper (1966).

Digestion of plant samples with Di-acid mixture. Powdered plant sample of 1.0 g were pre-digested with conc. HNO₃ overnight and then digested with di-acid mixture containing HNO₃ and HClO₄ in the proportion of 9:4 till a snow white residue was obtained (Piper, 1966). The volume of the digest was made to 100 ml with distilled water and used for total elemental analysis as detailed below.

Total phosphorus. A known volume of the di-acid digest was taken for total phosphorus determination by vanadomolybdo phosphoric yellow colour method in nitric acid system as described by Piper (1966).

Total potassium. Five ml of the di-acid digest was diluted to 50 ml with distilled water and fed to a calibrated flame photometer. By comparing the flame photometer reading of the sample with the calibration curve of potassium, the per cent potassium in the plant sample was calculated (Piper, 1966).

Calcium and magnesium. Five ml of the di-acid digest was titrated against standard EDTA after adding necessary reagents required for calcium and calcium plus magnesium as described by Piper (1966).

Total sulphur. Sulphur present in di-acid digest of the plant material was determined by precipitating the sulphate with barium chloride and turbidity was measured at 420 nm using spectrophotometer as described by Page *et al.* (1982).

C. Plant growth and yield

Observations were taken on growth parameters of tomato like plant height, plant fresh and dryweight, root length, root fresh and dry weight. Tomato fruit was harvested regularly starting from 75 DAT and yield from different treatments was compared with control.

D. Statistical analysis

Web Agri. Statistical Package (WASP), developed by CCARI (Central Coastal Agriculture Research Institute), ICAR research complex, Goa, was used for the statistical analysis of data obtained from all the in-vitro experiments. Critical difference and standard error of mean was analysed to compare and interpret the results of different experiments.

EXPERIMENTAL RESULTS

A. Germination Percentage

The tomato seeds of variety Abhinav was treated with different native isolates of *Trichoderma* spp. and sown in pro-trays and were observed for germination percentage (Table 5). All *Trichoderma* isolates increased the germination of seeds significantly and recorded highest of 92.85 followed by 90% germination in the isolates GMV, PSV and SMV, respectively. While the control treatment recorded least of 82% and commercial isolate recorded 85.71% seed germination.

Nutrient status of soil 7days before *Trichoderma* treatment. As per analysis N, P and K content of the soil before treatment was 272.80, 41.33 and 170.40 kg ha⁻¹, respectively. Whereas, soil contained 4.20 and 2.90 C. mol kg⁻¹ of Ca and Mg, respectively and 12.70 mg kg⁻¹ of S., before imposing the treatment (Table 6).

Nutrient status of soil after *Trichoderma* treatment:

Major Nutrient Content. Nitrogen, one of the major nutrients showed no significant difference between isolates and treatments (Table 7). Among different treatments, seed treatment alone recorded highest of 270.10 kg ha⁻¹ by commercial isolate. The combination treatment (T₂) also showed on par result with 269.20 kg ha⁻¹ N content in soil and the control recorded least of 265.40 kg ha⁻¹ nitrogen content and was significantly lower than other treatments. Phosphorous (P), also recorded no significant difference among different isolates. The isolates, SDKD and commercial showed higher soil content of P (40.60 kg ha⁻¹) and GMV recorded least of 38.10 kg ha⁻¹, while on par P content of 38.20 kg ha⁻¹ was noticed in control treatment. However, with respect to P content, treatments showed significant difference with highest of 40.60 kg ha⁻¹ in T₁ as against lowest of 38.20 kg ha⁻¹ in control treatment. The treatments and isolates showed no significant difference with respect to potassium (K) content also. Highest content of K was recorded by commercial isolate in combination treatment (T₂, 167.70 kg ha⁻¹) and least by control treatment (165.25 kg ha⁻¹).

In case of Ca and Mg, a significant difference among isolates and treatments was observed. The commercial isolate in T₁ showed greater Ca content of 4 m. eq. 100g⁻¹, However, we observed a least of 3.56 m. eq. 100g⁻¹ Ca content without *Trichoderma* treatment. again commercial isolate showed more Mg content in soil (2.60 m. eq. 100g⁻¹) in T₁ followed by 2.50 m. eq. 100g⁻¹ in T₂ and T₃, while other treatments were significantly superior than control treatment which recorded 2.15 m. eq. 100g⁻¹ Mg content. When we look into Sulphur status of soil after *Trichoderma* treatment, no significant difference was seen between both isolates and treatments, where 12 mg kg⁻¹ of S was recorded by commercial isolate in T₁ and least of 11.15 mg kg⁻¹ in control treatment which was significantly least than *Trichoderma* treatments.

Nutrient status of tomato plants 30 days after transplanting

We have observed a significant difference in nutrient content of tomato plant tissue, as influenced by different *Trichoderma* isolates and treatments. We have analyzed the plants for 6 different nutrients 30 DAT.

Major nutrient content of plants. Content of six major nutrients in tomato plants was analyzed using scientific methods and the observed values were indicated in percentage (Table 8).

With respect to all the major nutrients, among different treatments, combination of seed treatment and soil application of *Trichoderma* (T₂) has shown significant results by increasing nutrient uptake by plants and considered as best treatment followed by soil application alone (T₃) and seed treatment alone (T₁) which were superior over control treatment.

When we look into Nitrogen content, among isolates GMV was the superior one, recorded highest of 2.53% followed by 2.46% by SMV in T₂, whereas control treatment recorded nitrogen content of 1.32% and commercial isolate recorded 2.25% N, which was significantly inferior than native isolates. However, in case of T₁ isolate GMV (1.87 per cent) and in T₃ isolate SMV (2.22%) are the best isolates. Similarly, in case of Phosphorous, isolates GMV and SMV were found to be significantly superior which recorded P content of 0.29% as against control (0.24%) and commercial isolate (2.25% P) by T₂. The isolate CPV was the best isolate in case of potassium (K), with highest K content of 2.46% followed by 2.44% by GMV where both are on par in the treatment T₂ and least by commercial isolate (1.84% K). Whereas, control treatment recorded 1.43% K content. In case of other treatments, isolates PSV (2.05%) and 2.31% by CPV were the best isolates in the treatments T₁ and T₃, respectively.

With respect to other three major nutrients viz. Ca, Mg and S, the isolates SDKD (1.59% Ca), GMV (0.80% Mg and 0.60% S) and CPV (0.80% Mg) were the superior ones in the treatment T₂, whereas control treatment and commercial isolate recorded 1.03, 0.55 and 0.33% and 1.25, 0.78 and 0.47 % Ca, Mg and S, respectively. And in the other 2 treatments, isolates SMV (1.35% Ca and 0.60% Mg) and SDKD and GMV (0.52% S) were the best isolates in T₁ and SMV (1.44% Ca), GMV (0.78 Mg and 0.55% S) and PSV (0.78% Mg) were identified as superior in T₃.

Similar results were obtained by Rudresha *et al.* (2005) where they reported that the native isolates of *Trichoderma* spp. were involved in solubilisation of insoluble tricalcium phosphate to varying extents and in pot culture studies with rock phosphate, increased P uptake was observed in plants treated with *T. harzianum* (PDBCTH 10) followed by *T. virens* (PDBCTVs 12) and *T. viride* (TV 97). Rasool *et al.* (2011) observed that when tomato was sown in *Trichoderma* spp. T and *T. harzianum* T969 fortified soil there was an increase in uptake of nutrients viz., N, P, K, C and Mg when applied to soil than seed treatment.

Pre-sowing with *T. harzianum* alone increased the shoot N, P, Ca, Mg and S (Bombiti *et al.*, 2011). 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). Uptake of P, K, Mg and Zn was greatly enhanced by *T. asperellum* CHF 78 isolate which produces cellulases, chitinases, indole acetic acid (IAA), proteases and siderophores (Ying *et al.*, 2018). Singh *et al.* (2014) observed that combination of *T. harzianum* isolates (BHU51 + BHU105) increased the mineral content of tomato (N, P, K, Ca, Mg, S, Zn, Cu, Mn and Fe) which was higher (40-50% more than control) in *Trichoderma* treatments than untreated control.

The native isolates of *Trichoderma* spp. were scored as per increase in nutrient uptake by different isolates (Table 9), the isolates SMV, PSV and GMV were the most effective isolates, respectively. The isolates

significantly increased the uptake of P, Ca, Mg, N and S.

When compared with commercial isolate, all the native isolates were effective in increasing nutrient uptake by tomato plants.

Tomato growth promotion by *Trichoderma* treatment. Observations were made on growth parameters of tomato viz., plant height, plant fresh and dry weight, root length, root fresh and dry weight and fruit yield at respective time intervals to know the influence of *Trichoderma* isolates in different treatments on growth and effective utilization of absorbed nutrients from soil which is influenced again by *Trichoderma*.

Plant growth parameters: Tomato growth parameters viz., plant height (in cm, at 15, 30, 45 and 90 DAT) (Table 11) and plant fresh and dry weight at 90 DAT (Table 10) was analyzed and observed that there was a significant difference between isolates and treatments. Among different treatments, combination treatment (T₂) showed significantly superior results, where at 15 DAT isolate GMV was the best isolate with 42cm plant height followed by 41.67cm by PSV and CPV isolates and height gradually increased with age of plant, where isolates GMV and SMV were the superior isolates with height of 171.67cm and 170cm, respectively as against 30cm plant height in control treatment at 15DAT and 111.67cm at maturity of crop. However, the commercial isolate was significantly inferior than native isolates, that recorded least plant height (38.33cm @15DAT and 133.33cm @90DAT by T₂). Apart from T₂, at 90 DAT, in the treatments T₁ and T₃, isolate SMV was the best one (141.67 and 161.67cm, respectively). So, as nutrient uptake has increased, plant height also drastically increased by *Trichoderma*. Similarly, in case of plant fresh and dry weight at maturity of crop (Table 11), combination of seed treatment and soil application (T₂) showed significantly superior results than other treatments with best isolate GMV (highest fresh weight of 260.43g and 51.35g of dry weight) followed by 225.53g and 42.25g fresh and dry weight, respectively by isolate PSV, while the commercial isolate recorded lesser values of 201.34g and 38.68g fresh and dry weight in T₂. And the control was significantly inferior than *Trichoderma* treatments with 146.51 and 24.85g, fresh and dry weight, respectively. In case of T₁ and T₃ also GMV was the superior isolate with 198.69g and 247.36g fresh weight and 30.72g and 42.89g dry weight, respectively as against 146.88 and 190.22g plant fresh weight and 25.11 and 30.96g dry weight in T₁ and T₃, respectively by commercial isolate.

Root growth parameters. In order to take observations on root length, root fresh and dry weight, the plants were uprooted at maturity of the crop (90 DAT) and tomato plant root was observed for various parameters (Table 12). With respect to all parameters, T₂ was significantly superior over other treatments and control. Abul *et al.* (2012) also observed enhanced production and reduction in fertilizer requirement through use of enriched *Trichoderma*.

Among different isolates, SMV found to be superior isolate with 29.63cm root length, 46.06g fresh weight and 13.14g dry weight of roots in T₂, followed by 29.33cm, 41.60g and 12.21g of root length, root fresh and dry weight, respectively by PSV. However, commercial isolate recorded 25.60cm root length, 26.15g root weight and 7.20g root dry weight in T₂ which is lower compared with *Trichoderma* isolates while control recorded the least of 18.51cm root length, 16.09g and 3.50g root fresh and dry weight, respectively.

In the treatment T₁, the isolate GMV was the best one with respect to root length (23.97cm), whereas, isolate PSV was the superior one in case of root fresh and dry weight (21.74g and 4.60g, respectively) and in T₃ isolate SMV noticed as best one with 28.70cm root length, 36.89g and 9.89g root fresh weight and dry weight, respectively.

Tomato fruit yield. Once the tomato effectively absorbed soil nutrients which was influenced by *Trichoderma*, there will be increase in plant growth and as a result tomato fruit yield will be increased. In this context, tomato yield was analyzed and compared with control and commercial isolate (Table 13). Both treatments and isolates showed significant result, in which combination treatment (T₂) was the superior treatment than other ones, with best isolate GMV (520g fruit yield per plant) followed by 501.67g by PSV compared to commercial isolate (480g fruit yield) and least was seen in control (320.33g fruit yield). Similarly, in T₁, GMV was the best one (368.33g yield) and in T₃ GMV and PSV were the best isolates with same fruit yield of 485g.

Similar results were observed by Rasool *et al.* (2011) where they observed that growth parameters viz., shoot length, shoot and root fresh and dry weight were significantly increased when sown in *Trichoderma* spp. T and *T. harzianum* T₉₆₉ fortified soil compared to control. Khoshmanzar *et al.* (2020) observed that inoculation with *T. asperellum* B1092 effectively increased growth of tomato shoot and root than control treatment. Different treatments viz., *T. longibrachiatum* KH (T₁), *T. longibrachiatum* MA (T₂), *T. harzianum* (T₃) increased shoot dry weight by 97.2, 17.2 and 18.96% compared to negative control (without fungi) and 32.81, 19.41, 20.42% compared to positive control (without fungi but with chemical fertilizer).

There was 100, 83.33, 88.89% and 83% seedling emergence was observed in *T. harzianum* strains Plant shield (commercial isolate), T₂₂, T₉₅ and control, respectively. The strain T₉₅ showed highest plant height (34.35 cm), shoot fresh weight, dry weight, root fresh and dry weight was also highest in T₉₅ (43.10, 4.68, 9.13 and 0.87g, respectively) compared to other treatments (Shanmugaiah *et al.*, 2009). Ozbay and Newman (2004) reported that tomato seed treatment with native isolates of *Trichoderma* spp. (T₈₅, M14 and M2_14) led to a significant increase in plant height (25.22 cm by M2_14 *Trichoderma* isolate, 28.28 cm by T₈₅ isolate compared to 22.8 cm in untreated control) as well as root length (10.5 cm by M14 *Trichoderma* isolate, 10.85 cm by T₈₅ and 10.05 cm in blank control) and root fresh/ dry weight (4.45g and 2.93g fresh and dry weight by T₈₅ compared to 1.91g and 0.91g fresh and dry weight by blank control).

Table 1: List of native isolates of *Trichoderma* spp. used in the study and its source.

Sr. No.	Isolate code	Isolate name	Species
1.	SMV	Sheep manure, V. C. Farm	<i>Trichoderma viride</i>
2.	SDKd	Saw dust, K. M. Doddi.	<i>Trichoderma longibrachiatum</i>
3.	GMV	Got manure, V. C. Farm	<i>Trichoderma harzianum</i>
4.	PSV	Paddy straw, V. C. Farm	<i>Trichoderma harzianum</i>
5.	CPV	Coir pith, V. C. Farm	<i>Trichoderma asperellum</i>
6.	Commercial	-	<i>Trichoderma viride</i>

Table 2: Different treatments used to study the influence of native isolates of *Trichoderma* spp. on nutrient use efficiency in tomato.

Sr. No.	Treatments	Description
1.	T ₁	Seed treatment with <i>Trichoderma</i> alone @5g/kg of soil
2.	T ₂	Seed treatment with <i>Trichoderma</i> @5g/kg of soil + soil application of <i>Trichoderma</i> (30g/kg soil, 10days before transplanting)
3.	T ₃	Soil application alone of <i>Trichoderma</i> alone (30g/kg soil, 10days before transplanting)
4.	T ₄	No <i>Trichoderma</i> application

Table 3: List of methods adopted for soil nutrient analysis.

Parameters	Methods	References
Avail. N (kg ha ⁻¹)	Alkaline potassium permanganate distillation method	Subbiah and Asija (1956)
Avail. P (kg ha ⁻¹)	Olsen's extractant method, Colorimetry using ascorbic acid reagent	Jackson (1973)
Avail. K (kg ha ⁻¹)	Ammonium acetate extractant method, Flame photometry	Jackson (1973)
Exch. Ca and Mg [c. mol. (p ⁺) kg ⁻¹]	Ammonium acetate extractant method, Versenate titration method	Jackson (1973)
Avail. S (mg kg ⁻¹)	CaCl ₂ extractant method, Turbidometry	Black (1934)

Table 4: List of methods followed for the analysis of plant samples for nutrient content.

Plant analysis		
Parameter	Method	Reference
Nitrogen	Kjeldahl digestion and distillation Method	Piper (1966)
Phosphorus	Diacid digestion and Colorimetry using vanadomolybdate reagent	Piper (1966)
Potassium	Flame Photometry	Piper (1966)
Calcium and Magnesium	Complexometry using versenate Solution	Piper (1966)
Sulphur	Turbidometry	Bardsley and Lancaster (1965)

Table 5: Influence of *Trichoderma* application on germination of tomato seeds.

Sr. No.	Isolates	Seed Germination(%)
1.	SMV	90.00
2.	SDKD	88.57
3.	GMV	92.85
4.	PSV	92.85
5.	CPV	91.42
6.	Commercial	85.71
7.	Control	82.00

Table 6: Nutrient status of soil 7 days before *Trichoderma* treatment.

Sr. No.	Nutrients	Mean value
1	N (kgha ⁻¹)	272.80
2	P (kgha ⁻¹)	41.33
3	K (kgha ⁻¹)	170.40
4	Ca (C. mol.kg ⁻¹)	4.20
5	Mg (C. mol.kg ⁻¹)	2.90
6	S (mgkg ⁻¹)	12.70

Table 7. Influence of native isolates of *Trichoderma* spp. on major nutrient status of soil 30 DAT.

Sr. No.	<i>Trichoderma</i> isolates	Treatments	Major nutrient content					
			N (kgha ⁻¹)	P (kgha ⁻¹)	K (kgha ⁻¹)	Ca (m. eq./100g)	Mg (m. eq./100g)	S (mg/ kg)
1.	SMV	T ₁	269.30	40.10	167.40	3.80	2.30	11.60
		T ₂	268.10	39.40	166.80	3.60	2.20	11.70
		T ₃	268.40	39.60	167.00	3.80	2.30	11.70
		T ₄	265.40	38.20	165.25	3.56	2.15	11.15
2.	SDKD	T ₁	267.10	40.60	167.00	3.80	2.50	11.90
		T ₂	268.30	39.60	166.60	3.70	2.40	11.90
		T ₃	268.10	39.30	166.10	3.75	2.10	11.70
		T ₄	265.40	38.20	165.25	3.56	2.15	11.15
3.	GMV	T ₁	265.10	38.70	165.00	3.50	2.40	11.50
		T ₂	265.80	38.10	164.40	3.50	2.40	11.40
		T ₃	265.70	38.10	164.10	3.30	2.10	11.20
		T ₄	265.40	38.20	165.25	3.56	2.15	11.15
4.	PSV	T ₁	267.10	39.10	166.60	3.70	2.40	11.80
		T ₂	266.60	38.70	166.00	3.80	2.20	11.70
		T ₃	265.90	38.80	165.70	3.70	2.20	11.80
		T ₄	265.40	38.20	165.25	3.56	2.15	11.15
5.	CPV	T ₁	266.30	39.10	166.00	3.60	2.50	11.70
		T ₂	268.70	39.80	165.30	3.60	2.30	11.50
		T ₃	268.10	39.50	165.30	3.70	2.50	11.70
		T ₄	265.40	38.20	165.25	3.56	2.15	11.15
6.	Commercial	T ₁	270.10	40.60	168.10	4.00	2.60	12.00
		T ₂	269.20	40.10	167.70	3.75	2.50	11.70
		T ₃	268.30	39.80	167.40	3.80	2.50	11.90
		T ₄	265.40	38.20	165.25	3.56	2.15	11.15
7.	F	Isolate	NS	NS	NS	**	**	NS
		Treatments	NS	**	NS	**	**	
		Interaction (I*T)	NS	NS	NS	NS	NS	NS
8.	SEm ±	Isolate	4.26	0.60	2.63	0.05	0.04	0.18
		Treatments	3.48	0.49	2.15	0.04	0.03	0.15
		Interaction (I*T)	8.51	1.19	5.26	0.11	0.07	0.36
9.	CD @ 1%	Isolate	16.14	2.26	9.98	0.21	0.13	0.68
		Treatments	13.18	1.84	8.15	0.17	0.11	0.56
		Interaction (I*T)	32.29	4.52	19.95	0.41	0.26	1.36

**-. Significant, NS- Non-significant @ 1% level, I- Isolate, T- Treatment, I*T- Interaction.

T₁	Seed treatment with <i>Trichoderma</i> isolates @ 5gkg ⁻¹ of seed
T₂	Combination of seed treatment with <i>Trichoderma</i> isolates @ 5gkg ⁻¹ of seed+ soil application of <i>Trichoderma</i> isolates @ 30gkg ⁻¹ of soil
T₃	Soil application of <i>Trichoderma</i> isolates @ 30gkg ⁻¹ of soil
T₄	Control (no <i>Trichoderma</i> application)

Table 8: Influence of native isolates of *Trichoderma* spp. on major nutrient status oftomato plant 30 DAT.

Sr. No.	<i>Trichoderma</i> isolates	Treatments	Major nutrient content (%)					
			N	P	K	Ca	Mg	S
1.	SMV	T1	1.68	0.24	1.56	1.35	0.60	0.46
		T2	2.46	0.29	1.72	1.55	0.76	0.56
		T3	2.22	0.27	1.58	1.44	0.74	0.52
		T4	1.32	0.24	1.43	1.03	0.55	0.33
2.	SDKD	T1	1.77	0.27	1.59	1.08	0.57	0.52
		T2	2.44	0.28	1.74	1.59	0.68	0.55
		T3	2.11	0.27	1.62	1.17	0.66	0.54
		T4	1.32	0.24	1.43	1.03	0.55	0.33
3.	GMV	T1	1.87	0.26	1.44	1.15	0.59	0.52
		T2	2.53	0.29	2.44	1.25	0.80	0.60
		T3	2.05	0.25	1.68	1.18	0.78	0.55
		T4	1.32	0.24	1.43	1.03	0.55	0.33
4.	PSV	T1	1.74	0.25	2.05	1.14	0.56	0.46
		T2	2.52	0.27	2.36	1.55	0.79	0.54
		T3	2.19	0.26	2.21	1.25	0.78	0.49
		T4	1.32	0.24	1.43	1.03	0.55	0.33
5.	CPV	T1	1.72	0.24	1.73	1.06	0.59	0.43
		T2	2.34	0.27	2.46	1.32	0.80	0.48
		T3	1.99	0.25	2.31	1.15	0.76	0.45
		T4	1.32	0.24	1.43	1.03	0.55	0.33
6.	Commercial	T1	1.71	0.25	1.54	1.14	0.58	0.42
		T2	2.25	0.27	1.84	1.24	0.78	0.47
		T3	1.93	0.26	1.75	1.13	0.73	0.44
		T4	1.32	0.24	1.43	1.03	0.55	0.33
F	Isolate	**	**	**	**	**	**	
	Treatments	**	**	**	**	**	**	
	(I*T)	**	**	**	**	**	**	
SEm ±	Isolate	0.02	0.0008	0.01	0.01	0.17	0.00	
	Treatments	0.02	0.0007	0.01	0.01	0.14	0.00	
	(I*T)	0.05	0.0020	0.02	0.02	0.35	0.01	
CD @ 1%	Isolate	0.09	0.0000	0.04	0.05	0.66	0.01	
	Treatments	0.07	0.0000	0.03	0.04	0.54	0.01	
	(I*T)	0.18	0.0100	0.08	0.09	1.33	0.03	

** - Significant, NS - Non-significant @ 1% level, I - Isolate, T - Treatment, I*T - Interaction.

T1	Seed treatment with <i>Trichoderma</i> isolates @ 5gkg ⁻¹ of seed
T2	Combination of seed treatment with <i>Trichoderma</i> isolates @ 5gkg ⁻¹ of seed+ soil application of <i>Trichoderma</i> isolates @ 30gkg ⁻¹ of soil
T3	Soil application of <i>Trichoderma</i> isolates @ 30gkg ⁻¹ of soil
T4	Control (no <i>Trichoderma</i> application)

Table 9: Uptake of different nutrients enhanced by application of native isolates of *Trichoderma* spp.

Sr. No.	Isolates	Nutrients increased
1.	SMV	P, N, Ca, Mg, S
2.	SDKd	Ca, N
3.	GMV	N, P, K, Mg, S
4.	PSV	N, P, K, Ca, Mg
5.	CPV	S
6.	Commercial	---

Table 10: Influence of native isolates of *Trichoderma* spp. on tomato plant fresh and dryweight (g) at 30 and 90 DAT.

Sr. No.	<i>Trichoderma</i> Isolates	Plant fresh weight at 90 DAT				Plant dry weight at 90 DAT			
		Treatments				Treatments			
1.	SMV	T1	T2	T3	T4	T1	T2	T3	T4
1.	SMV	171.35	215.34	201.72	146.51	27.33	39.86	38.57	24.85
2.	SDKD	146.17	198.77	178.95	146.51	24.96	38.54	30.60	24.85
3.	GMV	198.69	260.43	247.36	146.51	30.72	51.35	42.89	24.85
4.	PSV	149.11	225.53	214.68	146.51	29.51	42.25	38.24	24.85
5.	CPV	145.65	175.26	170.85	146.51	24.88	33.12	30.50	24.85
6.	Commercial	146.88	201.34	190.23	146.51	25.11	38.68	30.96	24.85
7.	Mean	159.64	212.78	200.63	146.51	27.08	40.63	35.29	24.85
8.	I	T	I*T		I	T	I*T		
9.	F	**	**	**	**	**	NS		
10.	SEm ±	1.37	1.12	2.75		1.08	0.88	2.16	
11.	CD @1%	5.21	4.26	10.42		4.1	3.35	8.2	

** - Significant, NS - Non-significant @ 1% level, I - Isolate, T - Treatment, I*T - Interaction.

T1	Seed treatment with <i>Trichoderma</i> isolates @ 5gkg ⁻¹ of seed
T2	Combination of seed treatment with <i>Trichoderma</i> isolates @ 5gkg ⁻¹ of seed+ soil application of <i>Trichoderma</i> isolates @ 30gkg ⁻¹ of soil
T3	Soil application of <i>Trichoderma</i> isolates @ 30gkg ⁻¹ of soil
T4	Control (no <i>Trichoderma</i> application)

Table 11: Influence of native isolates of *Trichoderma* spp. on plant height (cm) of tomato at different intervals.

Sr. No.	<i>Trichoderma</i> isolates	Plant height (cm)															
		15 DAT				30 DAT				45 DAT				90 DAT			
		Treatments				Treatments				Treatments				Treatments			
T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4		
1.	SMV	34.67	39.67	37.33	30.00	74.67	83.83	79.67	58.50	104.33	118.67	115.33	87.53	141.67	170.00	161.67	111.67
2.	SDKD	34.17	38.67	37.67	30.00	71.33	84.17	79.33	58.50	104.67	121.17	116.50	87.53	120.00	155.00	138.33	111.67
3.	GMV	35.67	42.00	38.33	30.00	74.00	88.00	82.00	58.50	100.50	116.33	114.83	87.53	128.33	165.00	141.67	111.67
4.	PSV	36.67	41.67	39.33	30.00	77.00	86.33	83.67	58.50	105.83	122.83	118.17	87.53	140.00	171.67	153.33	111.67
5.	CPV	32.67	41.67	36.00	30.00	65.67	78.83	78.33	58.50	101.00	116.50	112.83	87.53	123.33	141.67	128.33	111.67
6.	Commercial	32.00	38.33	37.33	30.00	70.17	83.67	79.00	58.50	99.50	115.17	112.53	87.53	121.67	133.33	145.50	111.67
7.	Mean	34.31	40.33	37.67	30.00	72.14	84.14	80.33	58.50	102.64	118.50	114.97	87.53	129.17	156.11	144.81	111.67
8.		I	T	I*T		I	T	I*T		I	T	I*T		I	T	I*T	
9.	F	**	**	NS		**	**	**		**	**	NS		**	**	**	
10.	SE m ±	0.49	0.40	0.98		0.57	0.46	1.13		0.72	0.59	1.43		2.00	1.63	4.00	
11.	CD @1%	1.85	1.51	3.71		2.14	1.74	4.29		2.72	2.22	5.44		7.58	6.19	15.17	

** - Significant, NS - Non-significant @ 1% level, I - Isolate, T - Treatment, I*T - Interaction.

T₁ Seed treatment with *Trichoderma* isolates @ 5gkg⁻¹ of seed

T₂ Combination of seed treatment with *Trichoderma* isolates @ 5gkg⁻¹ of seed+ soil application of *Trichoderma* isolates @ 30gkg⁻¹ of soil

T₃ Soil application of *Trichoderma* isolates @ 30gkg⁻¹ of soil

T₄ Control (no *Trichoderma* application)

Table 12: Influence of native isolates of *Trichoderma* spp. on tomato root length (cm), root fresh and dry weight (g) after harvesting (90DAT).

Sr. No.	<i>Trichoderma</i> Isolates	Root length (cm)				Root fresh weight (g)				Root dry weight (g)			
		Treatments				Treatments				Treatments			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
1.	SMV	22.07	29.63	28.70	18.51	17.22	46.06	36.89	16.09	4.12	13.14	9.89	3.50
2.	SDKD	20.87	26.07	22.90	18.51	16.84	24.76	21.36	16.09	3.98	6.60	5.96	3.50
3.	GMV	23.97	29.03	24.17	18.51	17.02	28.99	19.88	16.09	4.03	11.98	5.09	3.50
4.	PSV	22.63	29.33	25.23	18.51	21.74	41.60	26.26	16.09	4.60	12.21	9.70	3.50
5.	CPV	21.60	24.83	22.23	18.51	19.15	27.68	21.96	16.09	4.56	8.15	7.54	3.50
6.	Commercial	21.07	25.60	22.13	18.51	15.07	26.15	21.57	16.09	3.59	7.20	7.05	3.50
	Mean	22.03	27.42	24.23	18.51	17.84	32.54	24.65	16.09	4.15	9.88	7.54	3.50
		I	T	I*T		I	T	I*T		I	T	I*T	
	F	**	**	**		**	**	**		**	**	**	
	S. E. m. ±	0.24	0.19	0.48		0.56	0.46	1.13		0.10	0.08	0.20	
	CD @1%	0.90	0.74	1.81		2.13	1.74	4.27		0.38	0.31	0.75	

** - Significant, NS - Non-significant @ 1% level, I - Isolate, T - Treatment, I*T - Interaction.

T ₁	Seed treatment with <i>Trichoderma</i> isolates @ 5gkg ⁻¹ of seed
T ₂	Combination of seed treatment with <i>Trichoderma</i> isolates @ 5gkg ⁻¹ of seed+ soil application of <i>Trichoderma</i> isolates @ 30gkg ⁻¹ of soil
T ₃	Soil application of <i>Trichoderma</i> isolates @ 30gkg ⁻¹ of soil
T ₄	Control (no <i>Trichoderma</i> application)

Table 13: Tomato fruit yield as influenced by native isolates of *Trichoderma* spp. (g plant⁻¹)

Sr. No.	<i>Trichoderma</i> isolates	Treatments				Mean
		T1	T2	T3	T4	
1.	SMV	351.67	491.67	483.33	320.33	442.22
2.	SDKD	325.00	484.00	472.33	320.33	427.11
3.	GMV	368.33	520.00	485.00	320.33	457.78
4.	PSV	345.00	501.67	485.00	320.33	443.89
5.	CPV	321.67	483.33	450.33	320.33	418.44
6.	Commercial	323.33	480.00	443.00	320.33	415.44
7.	Mean	339.17	493.44	469.83	320.33	
8.		Isolates (I)		Treatments (T)		Interaction (I*T)
9.	F	**		**		NS
10.	S. E. m. ±	5.69		4.65		11.39
11.	CD @1%	21.60		17.64		43.20

** - Significant, NS - Non-significant @ 1% level, I - Isolate, T - Treatment, I*T - Interaction.



Fig. 1. Mass multiplication of native isolates of *Trichoderma* spp. **A)** potato dextrose broth (PDB) medium preparation, **B)** Fully grown *Trichoderma* isolates on PDB medium in **1)** SMV, **2)** SDKd, **3)** Commercial, **4)** GMV, **5)** PSV, **6)** CPV, **C)** *Trichoderma* spore suspension, **D&E)** Mixing and drying of *Trichoderma* suspension with sterile talc powder.



Fig. 2. Raising of tomato seedlings for pot studies on influence of native isolates of *Trichoderma* spp. on nutrient use efficiency in tomato.



Fig. 3. Tomato plant growth in different treatments with the native isolate GMV (*T. harzianum*) at 30 days after transplanting



Fig. 4. Taking observations on height of tomato plants influenced by application of native isolates of *Trichoderma* spp.



Fig. 5. General view of the experimental setup (at 45 days after transplanting, **A&C**, **B**- giving support to tomato plants) of the study on influence of native isolates of *Trichoderma* spp. on nutrient use efficiency in tomato.

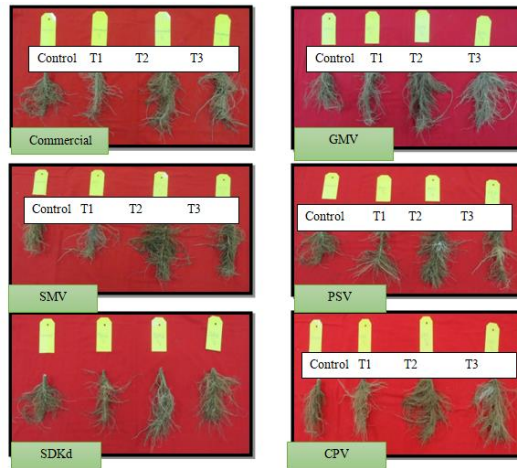


Fig. 6. A) Effect of native isolates of *Trichoderma* spp. on tomato root growth in different treatments at 90 DAT, **B)** measuring of root length in 1) control treatment, 2) *Trichoderma* treatment.

CONCLUSIONS

Application of 5 different non-rhizospheric native isolates in different treatments revealed T₂, the combination treatment as the best which recorded significant increase nutrient uptake, growth parameters and yield parameters of tomato plant. Among different isolates, SMV and PSV were the best isolates which recorded increase in uptake of maximum number of nutrients. The isolates SMV and PSV increased the uptake of P, Ca, K, N, Mg, and S. The isolate PSV was the best isolate which promoted all studied growth parameters. The isolate SMV was the next effective isolate with respect to tomato growth promotion. While, the isolate GMV showed superiority with tomato fruit yield. Thus, apart from understanding that the application of *Trichoderma* spp. proved benefit for the plant, it is evident that *Trichoderma* isolates obtained from non-rhizospheric sources can perform better and therefore is an important aspect for future research.

FUTURE SCOPE

In general, *Trichoderma* is isolated and mass multiplied in some location and their application will be done in some other locations which may leads to failure/reduction of BCA in its performance/antagonistic activity. So we isolated *Trichoderma* from local, non-rhizospheric sources and evaluated for antagonistic efficiency, plant growth promotion, compatibility with agrochemicals etc. which helps for identification of superior native isolates. we evaluated plant growth promotion and biocontrol activity under greenhouse conditions using pot culture technique. Further, effective isolates can be evaluated in field conditions and biochemical, enzymatic analysis can be done to study the mechanism of plant growth promotion and antagonism.

Author contribution. The work was done by Ajith C R, under the chairmanship of Dr. Pankaja N S who formulated the objectives and designed the work. All other authors provided necessary information and facilities to conduct the research work.

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Conflict of Interest. None.

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