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Enhancement of Drought Tolerance and Plant Growth in Tomato by *Trichoderma harzianum* Seed Treatment

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The proficiencies of soil microorganisms to drought tolerance have been known for many years, but their use as crop inoculants was not explored in agriculture commercially. One possibility to increase the drought tolerance is to use beneficial microorganisms as seed treatment. This study was conducted to investigate the effect of *Trichoderma harzianum* on the growth and development of Tomato (*Solanum lycopersicum* L.) plant under water stress condition. *Trichoderma harzianum* is applied to the seeds by treating seeds with *Trichoderma harzianum* before sowing. Parameters including germination percentage, chlorophyll content, leaf area, number of leaves, plant height, root length, plant fresh and dry weight and yield were recorded in all the treatments along with the untreated control. Tomato seeds treated with *Trichoderma harzianum* had higher seed germination

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percentage and chlorophyll content 95.50%, (2.41%), 42.59 SAPAD units (13.83%) respectively than untreated seed. Maximum leaf area and number of leaves 11.71 cm² (19.40%), 190.75 (15.43%) respectively recorded in the plants of *Trichoderma harzianum* treated seeds in water stress condition as compared to untreated seeds. Under extreme water stress condition plant height and root length of tomato plant increased significantly 98.25 cm, (19.45%), 41.25 cm (42.24%) respectively in *Trichoderma harzianum* treated seeds as compare to untreated seed. Also, *Trichoderma harzianum* seed treatment in tomato showed a significantly higher plant fresh and dry weight 189.43 g (8.65%) and 31.98 g (47.57%) respectively in drought condition as compared to untreated seeds. *Trichoderma harzianum* seed treatment played critical role in drought condition by increasing yield 731.25 g/plant (27.62%) increase over untreated seeds. Taken together, the study recommends that in drought condition for enhanced tomato growth *Trichoderma harzianum* should be used as eco-friendly solutions by seed treatment.

Keywords: Drought tolerance; growth parameters; tomato; seed treatment; root and shoot length; plant fresh and dry weight; Trichoderma herzianum; chlorophyll content and yield.

1. INTRODUCTION

"Climate change is a burning issue, and the incidence of global warming-induced by catastrophic climatic events is on the rise, causing global ecosystems including agroecosystems to suffer" [1]. "The occurrence of abiotic stresses like drought results in a considerable reduction in the yield of crop plants throughout the globe" [2]. "Concurrently, drought is becoming a dominant global environmental stressor for crop production, limiting food security with precarious economic and sociological impacts" [3]. "Due to climate change, water deficit is spreading to regions where drought was infrequent in the past" [4]. "Globally, the reduction in yield due to drought is likely to exceed the combined loss of all other possible causes of yield decline" [5,6]. Therefore, there is a need to identify solutions to mitigate water deficit stress and its impact on food security.

"The cultivation of tomato (*Solanum lycopersicum* L.) more than 7000 years ago in South America. Where small tomato fruits were first discovered and consumed orally and therapeutically. Tomatoes later spread to countries such as Peru, the Netherlands, and the United Kingdom, and are now grown and consumed in all parts of the world. There are many varieties of tomatoes and it is one of the most widely consumed vegetables in the world. Tomato is an annual plant and it is one of the most commercially important vegetable. Today, tomato is the second most consumed and widely grown nonstarchy vegetable in the world after potato. The average world production of tomato is estimated to be around 159 million tons. In the last few years, tomato consumption has increased considerably as they became an important part of the human diet. Therefore, the area of tomato cultivation has also expanded globally in the last few years. The production data shows that tomato production was 206.2 lakh tons in year 2022-23" (Spiralling Tomato Prices: Issues and Concerns, Issue No.: 03, August 2023, NABARD,).

"New and novel solutions for plant growth enhancements are required to ease the burden imposed on our environment and other resources. The relationship between plants and soil microbial communities is crucial to plant health" [7]. "Here, we look at potential solutions to these issues by investigating some of the research conducted regarding the biological inoculations of free-living plant growth promoting microorganisms. The genus *Trichoderma*, belonging to the Hypocreaceae family (Hypocreales, Sordariomycetes, Ascomycota), is a highly studied group of filamentous fungi known for its numerous beneficial traits, particularly in agriculture as key components of microbiological inoculants. According to the most recent taxonomic concept, more than 400 described species of the genus are divided into 8 main clades" [8]. "The beneficial effects of *Trichoderma*, however, are more pronounced when plants are under stress compared to when grown under optimal conditions" [9,10].

The purpose of the present study was to investigate the effects of *Trichoderma harzianum* seed treatment in drought conditions to enhance and promote yield and vegetative growth through the analysis of some indicating factors, such as germination percentage, chlorophyll content, leaf area, number of leaves, plant height, root length, plant fresh and dry weight and yield of tomato (*Solanum lycopersicum* L.) plant.

2. MATERIALS AND METHODS

2.1 Experimental Site and Materials

Investigation was carried out in greenhouse during 2023-2024 at Umergaon, District Valsad, Gujarat in tomato (*Solanum lycopersicum* L.) crop. The Namdhari-NH-585" indeterminate early maturing variety of Namdhari seeds was selected for carrying present experiment.

Trichoderma harzianum (WP)-61, trade name Neemoderma-H of Shri Ram Solvent Extract Extractions (P) Ltd. having *Trichoderma harzianum* content 1% w/w (CFU Count 2x10⁶/gm. Min) plus carboxy methyl cellulose 1.0% w/w and talc powder 98.0% w/w. was used for *Trichoderma harzianum* seed treatment.

Surface soil (0-15 cm) collected from farmer's field and mixed thoroughly after air dried and passed through 2 mm sieve. Well decomposed FYM is mixed in collected surface soil (10:1 ratio) and autoclaved at 15 PSI pressure and 121ºC temperature for 30 minutes to sterilize. Autoclaved soil is filled in 12-inch plastic pots at the rate of 6.3 kg autoclaved soil in each pot and pots are arranged in greenhouse.

2.2 Seed Treatment with *Trichoderma harzianum* and Seed Sowing

Tomato seeds are surface sterilized with 1% sodium hypochlorite for three minutes. Then treated seeds are washed with sterilized water and dried. The dried 30 seeds of tomato are treated with 1g *Trichoderma harzianum* in Petri plate (Fig. 1.). For untreated control seeds of tomato are soaked in distilled water. Seed treatment is given to tomato seeds as per treatment details (Table 1.). Single seed sown in each pot and pots are arranged in greenhouse. 500 ml water is applied to each pot at every alternate day with the help of measuring beaker till 15 days. 28ºC to 32ºC temperature and 55% to 60% relative humidity is maintained in respective greenhouse throughout the crop lifecycle (Fig. 2.).

2.3 Seed Germination Percentage

Seed germination percentage was calculated at 15 days after sowing. Seed germination percentage was calculated by using the following formula (ISTA, 2010):

Germination percentage = (Number of germinated seeds/Total number of seeds sown) X100.

Table 1. Treatment details of seed treatment

Treatment No.	Treatment Details
T1	*T. harzianum* + Irrigated
T2	*T. harzianum* + Water Stress
T3	Untreated seed + Irrigated
T4	Untreated seed + Water Stress (Control)

Fig. 1. Seed treatment with *Trichoderma harzianum*

Fig. 2. Overview of experiment in Greenhouse

2.4 Total Chlorophyll Content

Chlorophyll content was measured with the help of portable chlorophyll meter SPAD (Soil Plant Analytical Development) chlorophyll meter (MinoltaTM) at 90 days after sowing. It was used to acquire rapid estimation of chlorophyll content in SPAD units [11]. The measurement was done at morning 10 am. It helps to avoid droplets content on leaf surface.

2.5 Leaf Area and Number of Leaves

In tomato crop total number of leaves are counted after five picking/harvesting. Total leaf area was measured by using methods as given below:

Leaf area = L x W x F

where L = Maximum length (cm); W = Maximum width (cm); F = Factor (0.50)

2.6 Plant Height and Root Length

The plant height and root length were measured after five picking from each pot in each replication with the help of measuring scale.

2.7 Plant Fresh Weight

At the time of harvesting plants of tomato from each treatment were softly uprooted from pots and washed off soils from the roots by running water very carefully. Plants were dried by blotting paper for removing surface moisture. Then, shoots were cut at soil line and separated. Fresh weight of root and shoot were measured on electronic balance up to three decimal digits and plant fresh weight recorded.

2.8 Plant Dry Weight

Dry weight of tomato was measured at harvesting stage. Plants from each treatment were softly uprooted from pots. and washed off soils from the roots by running water very carefully. Plants were dried by blotting paper for removing surface moisture. Then the plants were taken in brown envelop and dried in an oven set to low heat (100°F) overnight. After that, plants were cooled in a dry environment. Then, shoots were cut at soil line and separated. Shoots and root of each plant separately weighed and plant dry weight recorded.

2.9 Yield

The matured tomatoes are harvested and weight of tomato was recorded on electronic digital balance. Yield of five subsequent picking is considered in tomato for yield calculation.

2.10 Statistical Analysis

The data generated from the pot culture experiment was analyzed by completely randomized design. The data obtained was statistically analyzed and appropriately interpreted as per the methods described in "Statistical Methods for Agricultural Workers" by Panse and Sukhatme, [12], Appropriate standard error (S.E.) and critical differences (C.D.) at 5% level were worked out as and when necessary and used for data interpretation. The data were statistically.

3. RESULTS AND DISCUSSION

3.1 Seed Germination Percentage

Trichoderma harzianum treated seed of tomato has 95.50 to 94.75 percent seed germination, which is statistically similar with other all treatments, however numerically higher than the untreated seed 93.75 to 93.25 percentage (Fig. 3) (Table 2). Same results are noted by Donoso et al., [13] that inoculation of tomato seed with *Trichoderma* improved germination and other growth parameters when grown at water deficit conditions. Shoresh et al*.,* [14] found that some species of this *Trichoderma* have direct effects on plants, increasing their potential for growth and nutrient absorption, fertilizer efficiency, a higher rate and percentage of seed germination, and stimulating plant defense against biotic and abiotic damage. Mastouri et al*.,* [15] observed similar results that *Trichoderma harzianum* added as seed treatment (tomatoes) or as a soil treatment (Arabidopsis) largely improved the germination at osmotic potentials of up to 0.3 MPa. Plants grown from these *Trichoderma* treatments are much more resistant to water deficit conditions.

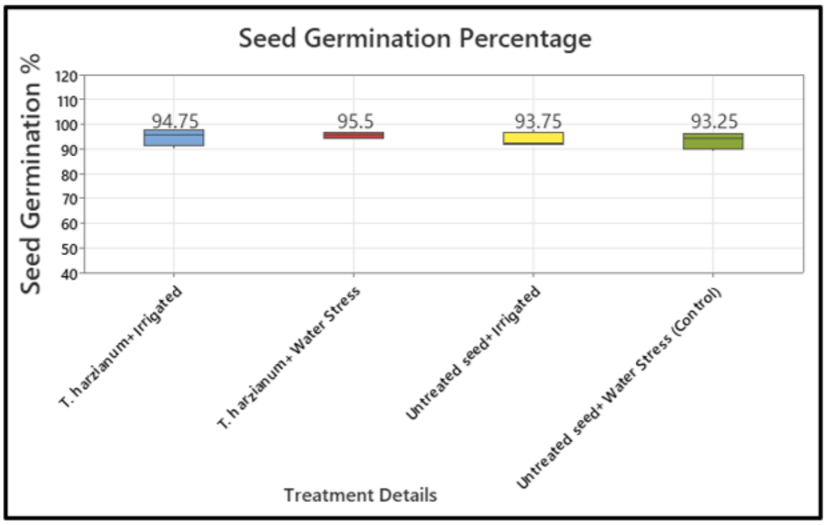


Fig. 3. Effect of seed treatment with *Trichoderma harzianum* on seed germination

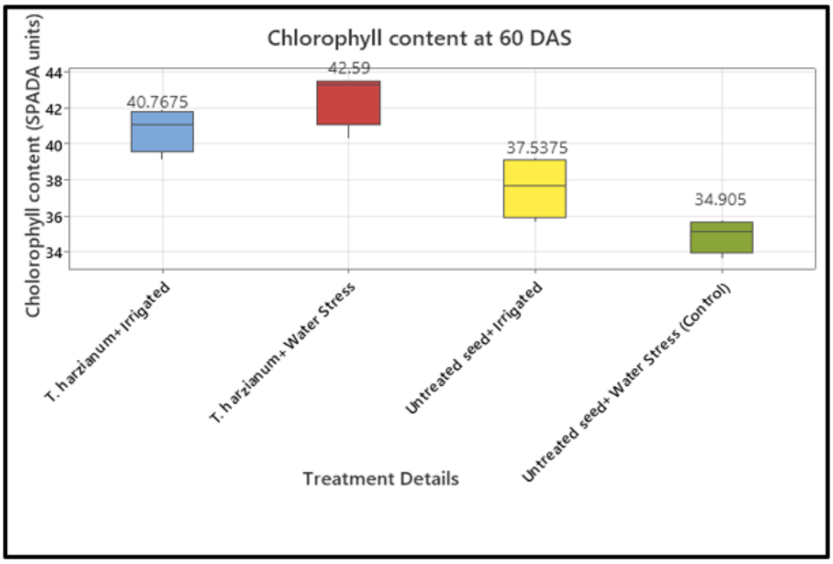


Fig. 4. Effect of seed treatment with *Trichoderma harzianum* chlorophyll content

3.2 Total Chlorophyll Content

Drought reduces the chlorophyll content severely in plant. We recorded significant results of *Trichoderma harzianum* seed treatment, the maximum chlorophyll content was recorded in the treatment T2 i.e. seed treatment of *Trichoderma harzianum* with water stress condition, which has (42.59 SPADA units) 22.02% increase over control followed by the T1 treatment plants i.e. seed treatment with *Trichoderma harzianum* with regular irrigation (40.77 SPADA units) 16.80% increase over control (Fig. 4) (Table 2). The T3 treatment i.e. untreated seed with regular irrigation has (37.54 SPADA units) 7.54 % increase over control. The control T4 treatment having untreated seed with water stress condition has 34.91 SPADA units chlorophyll content. Shukla et al*.,* [16] observed that *Trichoderma harzianum* inoculated plants exhibit delayed wilting, increased stomatal conductance, enhanced leaf chlorophyll content, and greater net photosynthesis levels under water deficit stress conditions. Contreras-Cornejo et al*.,* (2019) found that the plants inoculated with *Trichoderma* isolates can enhance water-deficit tolerance by improving root development, regulation of drought-induced changes in the stomatal opening, photosynthesis, and chlorophyll content in leaf.

3.3 Leaf Area and Number of Leaves

Significant variation of leaf area and no of leaves observed with different treatments in tomato plant (Fig. 5 and 6) (Table 2). Treatment T2 i.e. seed treatment with *Trichoderma harzianum* having water stress condition has found maximum leaf area 19.40% (11.71 cm^2) increase over control followed by T1 *Trichoderma harzianum* with mean leaf area of 11.12 cm^2 increasing leaf area by 13.38% over control. Untreated plants with regular irrigation i.e. T3 have 10.50 cm^2 leaf area which is a 7.01% increase over control. The control i.e. T1 untreated seed with water stress has 9.81 cm^2 leaf area. Rabeendran et al*.* [17] experimented the evaluation of *Trichoderma* isolates in a glasshouse to check the growth promotion effects of *Trichoderma* on cabbage seedlings (*Brassica oleracea* L.) and reported that increased ($P<0.05$) leaf area (58 - 71%), shoot dry weight (91 - 102%) and root dry weight (100 - 158%) compared with the untreated control.

Among all the treatments, T2 treatment i.e. seed treatment with *Trichoderma harzianum* having water stress condition shows higher number of leaves per plant (190.75), which is 15.43% over control followed by treatment T2 having *Trichoderma harzianum* seed treatment with regular irrigation recording 189.75 leaves per plant and 14.83% increase over control (Fig. 6) (Table 2). The treatment T3, i.e. untreated seed with regular irrigation have 185.25 leaves per plant having 12.10% increase over control. The untreated seed with water stress condition control has 165.25 leaves per plant. Sundaramoorthy and Balabaskar (2013), recorded similar results that there was an increase in the plant height, number of leaves, and total yield of tomatoes in plants treated with *T. harzianum.*

3.4 Plant Height and Root Length

In present study, we observed that drought reduces the growth drastically and *Trichoderma harzianum* not only improved these negative effects but also enhanced the plant growth significantly by increasing plant shoot and root growth (Fig. 7) (Table 2.). Furthermore, we observed that the tomato plants of treatment T2 has 98.25cm plant height which is 19.45% increase over control followed by treatment T1 with tomato plant height of 95.75 cm, which is 16.41% increase over control. The Treatment T3 has 92.75 cm height and 12.77% increase over control. The control plant has 82.25 cm height. Kucuk [18] conducted experiment on wheat and reported that seed treatment with *Trichoderma harzianum* will help to increase the considerable plant height.

Significant improvement in root length (41.25 cm) was achieved in the plants of treatment T2 with 42.24% increase over control. This treatment is followed by T1 by 29.31% (37.50cm) over control (Fig. 8) (Table 2). Treatment T3 enhanced the root length by 20.69% (35 cm) over control. Then, the control treatment T4 recorded 29 cm root length. Cai et al*.* [19] reported that harzianolide produced by *Trichoderma* spp. can improve the early stage of plant development enhancing the root length. These morphological modifications are possible because of the ability of the *Trichoderma* spp. to act through several mechanisms such as environmental buffering (against pH, drought, waterlogging, cold and heat), phosphorus solubilization, organic matter decomposition, chilation and siderophore production.

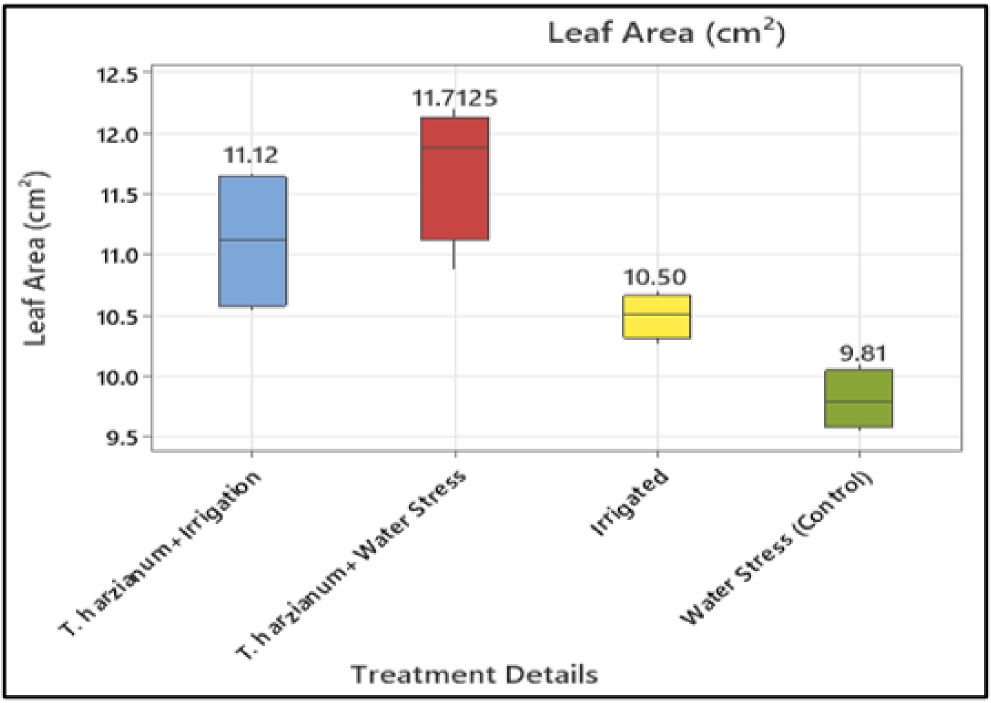


Fig. 5. Effect of seed treatment with *Trichoderma harzianum* on leaf area

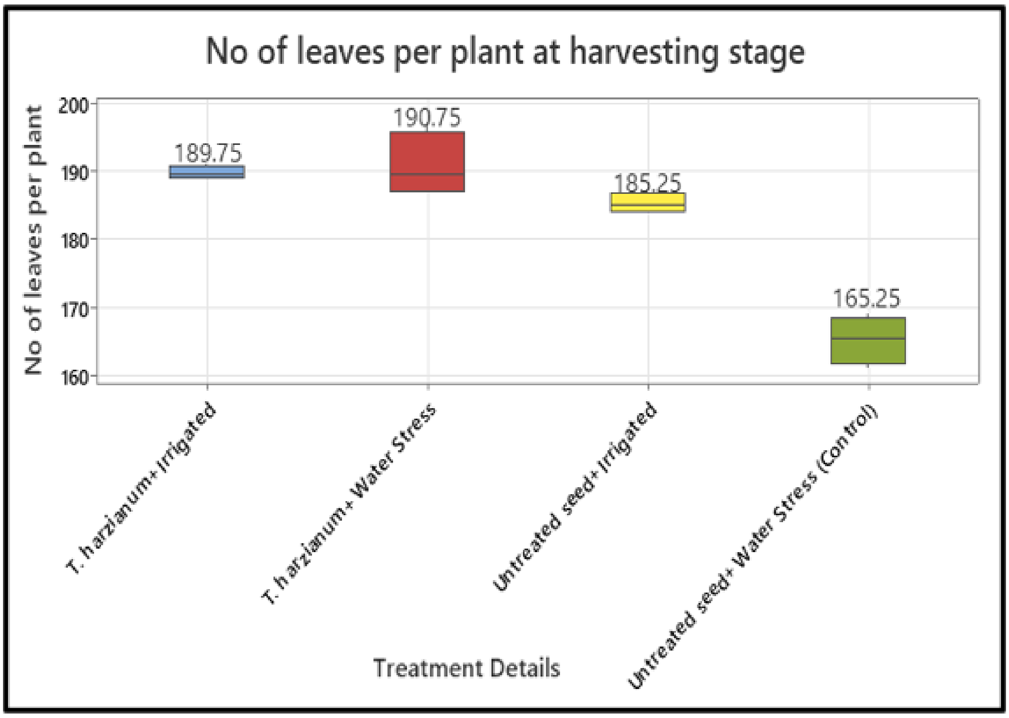


Fig. 6. Effect of seed treatment with *Trichoderma harzianum* on number of leaves

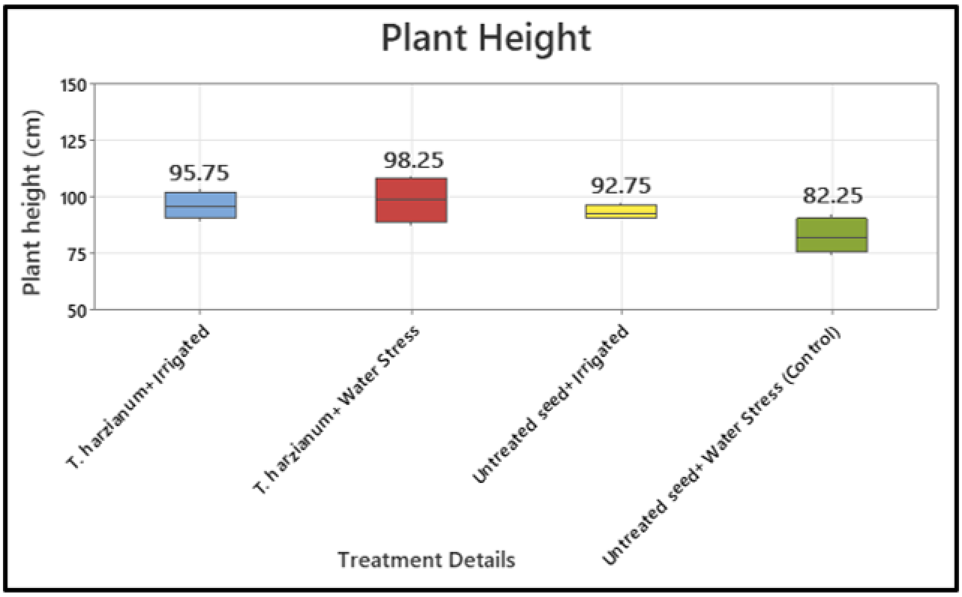


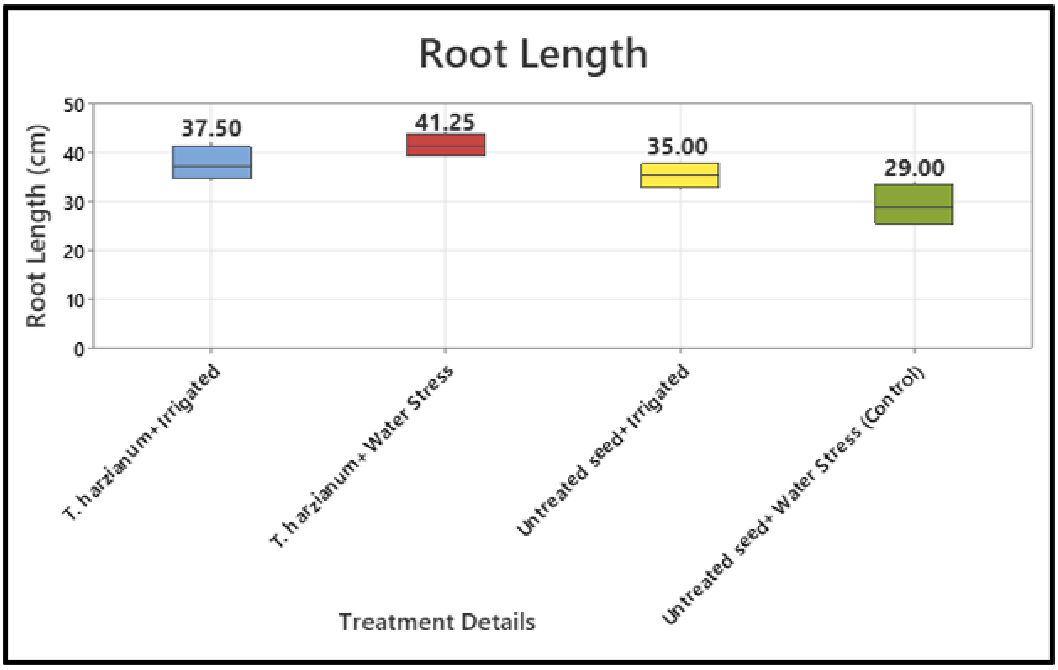
Fig. 7. Effect of seed treatment with *Trichoderma harzianum* on Plant height

3.5 Plant Fresh Weight

The data presented in Table 2 indicate that the T2 treatment has greater plant fresh weight 189.43 g, which is 8.65% increase over control followed by T1 treatment with 182.17 g and 4.49% increase over control (Fig. 9) (Table 2). The T3 treatment record 178.26 g of plant fresh weight, which is 2.245 increase over control and treatment T4 has 174.35 g plant fresh weight, which is comparatively less than other all treatment. Chacón et al. [20] found that inoculation of *T. harzianum* increased fresh weight and lateral roots in tomato plants. *Trichoderma* species are present in all soil types and are the most common cultivable fungi.

3.6 Plant Dry Weight

As like plant fresh weight observations we noticed that the T2 has a maximum plant dry weight of 31.98 g, which is 47.57% increase over control followed by treatment T1 with 29.09 g having 34.21% increase over control (Fig. 10) (Table 2). The treatment T3 has 26.88 g of plant dry weight which is 24.03% increase over control. In control treatment T4 the plant dry weight record is 21.67 g. Camargo et al., [21] reported that the application of commercial *Trichoderma* sp. in the cultivation of pea *Pisum sativum* L. (Fabaceae) significantly improved its growth and development, influencing physiological variables such as germination, leaf area, dry and fresh weight of the root, dry and fresh weight of the aerial part and length of root, favoring the productive yield of the crop, when applying the same treatment to the seed.



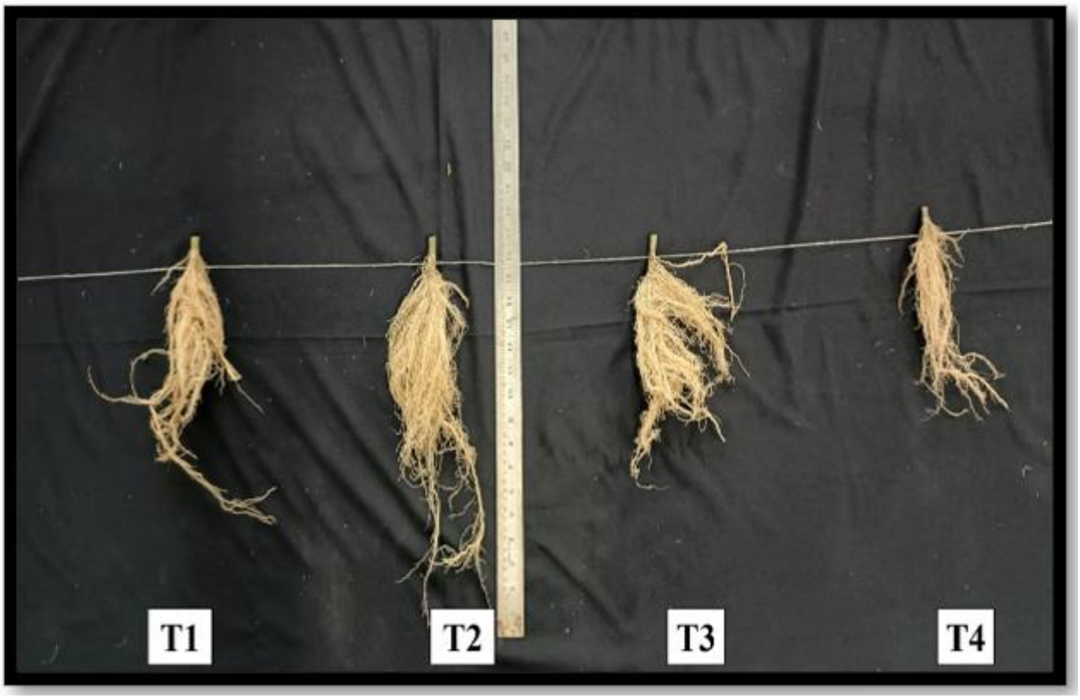


Fig. 8. Effect of seed treatment with *Trichoderm harzianum* on Root length

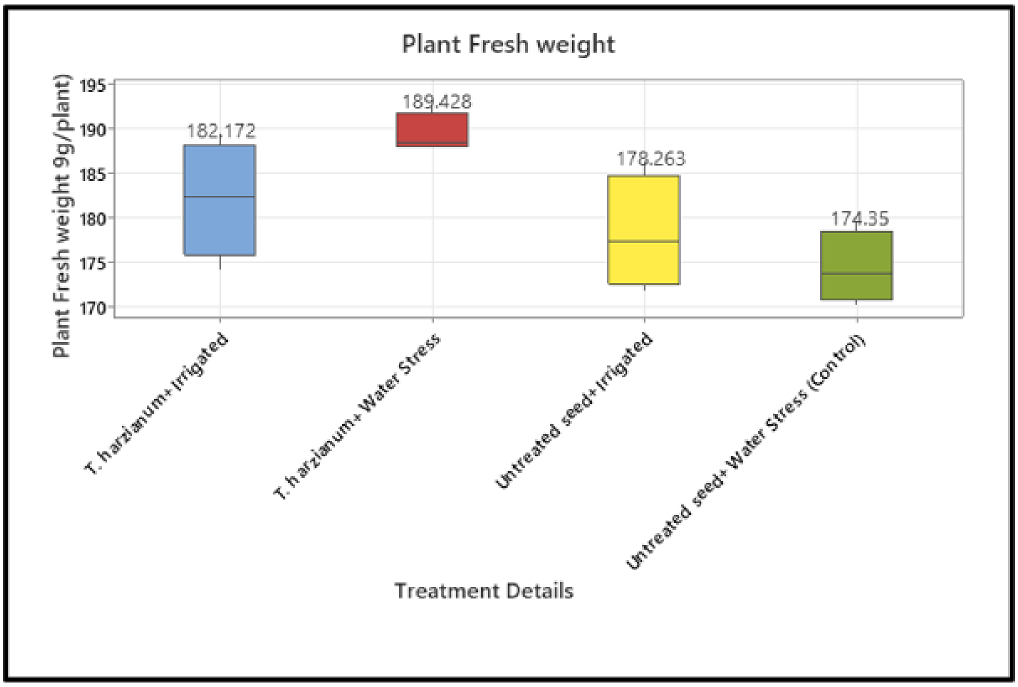


Fig. 9. Effect of seed treatment with *Trichoderma harzianum* on plant fresh weight

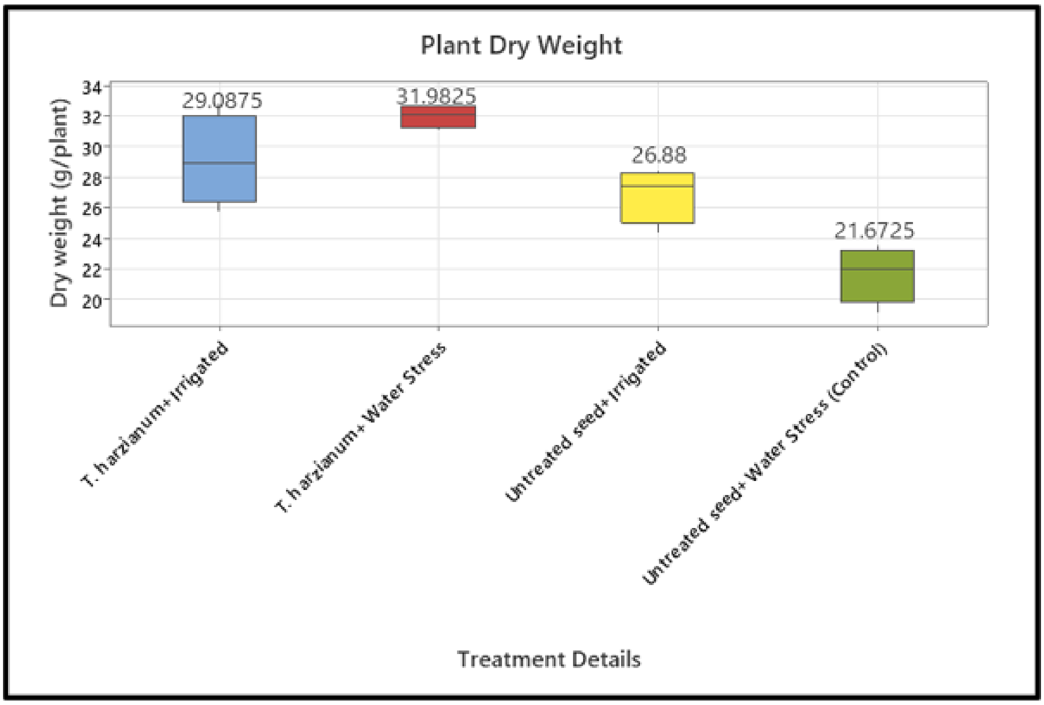
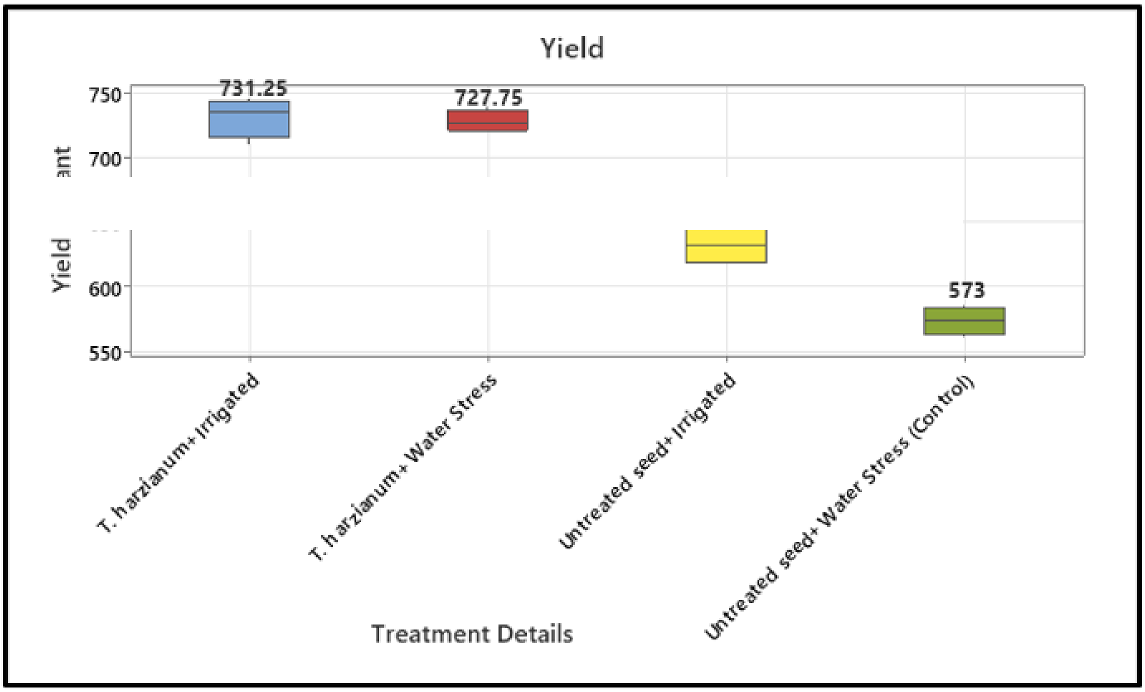


Fig. 10. Effect of seed treatment with *Trichoderma harzianum* on plant dry weight

3.7 Yield

Results indicate that *Trichoderma harzianum* seed treatment significantly increased the yield even in drought condition. The treatment T2 exhibit maximum yield of 731.25 g, which is 27.62% increase over control followed by T1 treatment with 727.75 g and 27.01% increase over control (Fig. 11) (Table 2). With the treatment T3 the yield recorded is 635.25 g with

10.86% increase over control. The control treatment T4 untreated seed with water stress condition has 573g yield. Mishra et al. [22] found that *Trichoderma* spp. seed treatment at sowing will boosts chlorophyll accumulation, root and shoot growth, tiller number, and crop yield in rice even under drought conditions. Scudeletti et al. [23] conducted field experiment in sugarcane crop and reported that *Trichoderma* spp. inoculation of sugarcane improves plant morphology and physiological factors under drought stress which contribute in increasing sugarcane yield [24,25].



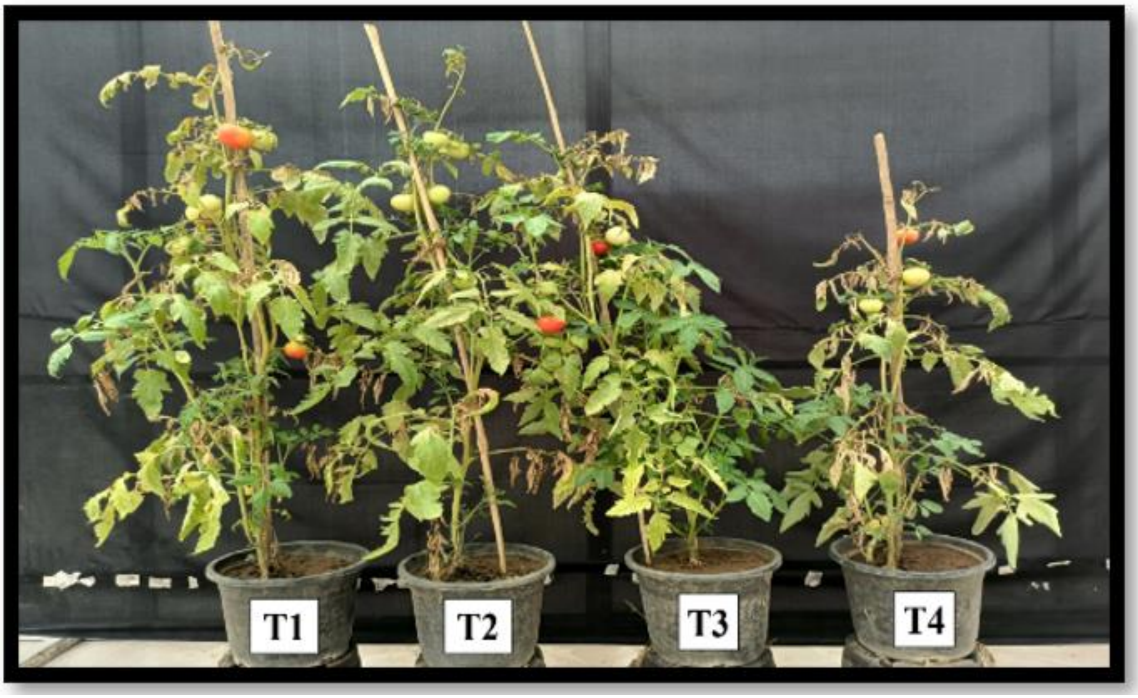


Fig. 11. Effect of seed treatment with *Trichoderma* Yield harzianum on Yield

Table 2. Effect of *Trichoderma harzianum* on Germination %, Chlorophyll content, Leaf area, No of leaf/plant, Plant height, Root length, Fresh and Dry weight and Yield

Trt.#	Treatment Details	Germination %	Chlorophyll content (SPADA units)	Leaf Area (cm)	No of Leaves (Nos.)	Plant Height (cm)	Root Length (cm)	Fresh Weight (g)	Dry Weight (g)	Yield (g)
T1	*T. harzianum* + Irrigated	94.75	40.77	11.12	185.25	95.75	37.50	182.17	29.09	727.75
T2	*T. harzianum* + Water Stress	95.50	42.59	11.71	190.75	98.25	41.25	189.43	31.98	731.25
T3	Untreated seed + Irrigated	93.75	37.54	10.50	189.75	92.75	35.00	178.26	26.88	635.25
T4	Untreated seed + Water Stress (Control)	93.25	34.91	9.81	165.25	82.25	29.00	174.35	21.67	573.00
	S.Em.±	**1.64**	**0.53**	**0.49**	**1.65**	**3.21**	**1.63**	**2.83**	**0.97**	**7.59**
	C.D. at 5 %	**NS**	**1.69**	**1.56**	**5.26**	**10.28**	**5.23**	**9.07**	**3.10**	**24.28**
	C.V. %	**3.48**	**2.72**	**2.46**	**1.80**	**6.96**	**9.15**	**3.13**	**7.08**	**2.46**

4. CONCLUSION

In farming, the novel and conventional inventions boost up the yield of agriculture produce. The challenge faced by modern agriculture is to accomplish high yields in an environment-friendly manner. Hence, quick action on finding eco-friendly solutions needs to be done.

It is worldwide well known that *Trichoderma harzianum* is used as a biocontrol agent against different plant pathogens. Existing study results indicate that seed treatment with *Trichoderma harzianum* provides an innovative, cost-effective, low toxicity and environmentally friendly means of increasing crop yields in tomato through improving seed germination, chlorophyll content and growth under abiotic stress like drought stress conditions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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