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Effectiveness of local isolates of *Trichoderma* spp. in imparting drought tolerance in rice, *Oryza sativa*

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Rice is a crop that requires high amount of water, and the drought is a major constraint in paddy cultivation. Water stress condition frequently prevails due to shortage of rain which results in significantly reduced plant growth and yield of rice. In the present study capability of *Trichoderma* spp. in imparting drought tolerance to rice, *Oryza sativa* was explored. Eleven local strains of *Trichoderma* spp. were applied to rice cv. Swarna Sub-1 through soil application (2 g/kg soil) and seed treatment (20 g/kg seed) under 0, 25, 50 and 75% less watering of the recommended amount. The soil application of *T. harzianum* AMUTHZ84 significantly promoted the shoot and root length (23.6 and 21.3%) followed by seed treatment (19.7 and 18.2%) under recommended level of irrigation condition (100% irrigation). Next in effectiveness was *T. viride* AMUTVR73 (21.5 and 18.1%) over untreated control. However, under 75% water availability, soil application with *T. harzianum* AMUTHZ82 was found superior over other isolates in enhancing shoot and root length (17.7 and 16.4%). The same isolate was also recorded to be superior under 50% (12.4 and 10.1%) and 25% water availability (9.3 and 8.1%) in enhancing the plant growth and biomass of rice cv. Swarna Sub-1. The isolate also significantly enhanced the leaf pigments, and photosynthesis in the rice plants grown under 25–75% water stress condition. In general, soil application of *Trichoderma* isolates was found more effective than seed treatment, and the *T. harzianum* AMUTHZ82 provided 8–17% enhancement in the plant growth, biomass, leaf pigments and photosynthesis of rice cv. Swarna Sub-1 grown under 25–75% water stress condition.

Rice is the primary and fundamental food source providing 20% of the global calories consumed by over half the world's population and fulfils the dietary requirements of a large proportion of the human population especially in Asia and Africa[1]. It is cultivated on 165.25 mh with an annual global production of around 787.29 mt [3]. India has the largest land area under rice cultivation, but occupies second place in the tonnage globally after China. This is largely due to lower productivity rate of rice in India (4.21 tons/ha) over China (7.11 tons/ha) and the rest of the world 4.76 tons/ha[2]. In India, rice is grown in all states. West Bengal is the highest rice-producing state (16.72 mt), while Punjab has highest productivity rate (5.10 ton/ha). Uttar Pradesh is the second largest producer of rice, where around 5.60 mha of land is used to grow rice, with a total production of around 15.2 mt[4].

Despite of being highest in rice acreage in the world, the annual gross productivity of rice in India is 40% less than China[2]. The significantly lower rice productivity rate in India is due to regular occurrence of biotic and abiotic stresses in rice cultivation[5]. Drought or lesser availability of water to paddy crop is a major and challenging abiotic stress to rice crop[5]. Rice plants require saturated or semi-saturated condition to maintain proper growth and development for better productivity. The crop yield is hampered severely when there is a limitation in the water availability[5,6]. The moderate drought can potentially reduce rice crop yields by up to 9–10% globally[7–9].

Trichoderma spp. are a group of filamentous fungi that colonize the rhizosphere in all natural habitates, and may also form symbiotic relationships with plants[7,11]. They are renowned for their multifunctional capabilities, including suppression of plant pathogens[2,12], promotion of plant growth, and modification of plant responses to abiotic stresses[13]. Several studies have demonstrated the positive impact of *Trichoderma* spp. in enhancing drought tolerance in rice[14,15]. Some species of *Trichoderma*, for example *T. harzianum* is an efficient mineral solubilizer and pathogen suppressor that can colonize the roots of crop plants and act as an efficient mycoparasite[16–18].

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The biopriming with *Trichoderma* spp. can enhance nutrient absorption and improves water-holding capacity[19], leading to gradual improvement in the plant growth and development under water stress conditions[20–22]. Besides the plant breeding for drought tolerance, no other reliable and effective technique has been developed to protect rice from water stress due to low rainfalls. In recent years potential of microorganisms in imparting tolerance against water stress has been evaluated[23]. *Trichoderma* spp. have demonstrated promising potential that can be exploited commercially to protect plants under moderate level of droughts[24,25]. *Trichoderma* spp. have been reported in reducing the impact of biotic and abiotic stress on plants[26,27]. The present study was carried out to examine and ascertain the role of *Trichoderma* spp. in protecting rice plants from the impact of water stress.

Results

Isolation and characterization of *Trichoderma* species

The DNA of *Trichoderma* species was sequenced by Macrogen® Incorporation, South Korea using universal primer (ex, 16S V3-V4, ITS, 18S) and Sanger sequencing method. The 16S and 18S rDNA gene sequences showed 99% similarity of the isolates with *Trichoderma* spp. (Fig. 1). The isolate showed 99 homologies with the ITS region of *Trichoderma* spp. Thus, the isolates were identified as *Trichoderma harzianum, T. viride, T. virens, T. hamatum* and *T. koningii.* The 16S and 18S rDNA gene sequences were deposited in NCBI gene bank under the accession number listed in Table 1. However, to avoid error in the grouping by ITS region, it is recommended to perform sequencing of the conserved region genes such as the TEF gene, Beta-tubulin gene, and Histone H3 gene for species-level identification in fungal isolates, especially, the TEF gene which shows a high level of sequence polymorphism among closely related species, even in comparison to the intron-rich portions of protein-coding genes such as the calmodulin gene, beta-tubulin gene, and histone H3 gene.

Plant growth

The rice cv. Swarna Sub-1 grown under water deficiency condition (75, 50 and 25% water availability of the recommended amount) showed the symptoms of water stress, exhibiting reduced plant growth, with shorter stems and smaller leaves in comparison to plants received the recommended level of irrigation. The symptoms varied with the level of water stress. In general, the rice cv. Swarna Sub-1 was found highly sensitive to drought (water stress). However, the soil application of *T. harzianum* AMUTHZ82 subsided the water stress symptoms and promoted shoot and root length by 19.7 and 16.8% with 75% watering, 17.4 and 14.1% with 50% watering and 12.3 and 11.2% with 25% watering over respective controls (Tables 2, 3). Next in effectiveness was *T. viride* AMUTVR73, other isolates did not produce the significant effect. The seed treatment also increased the length

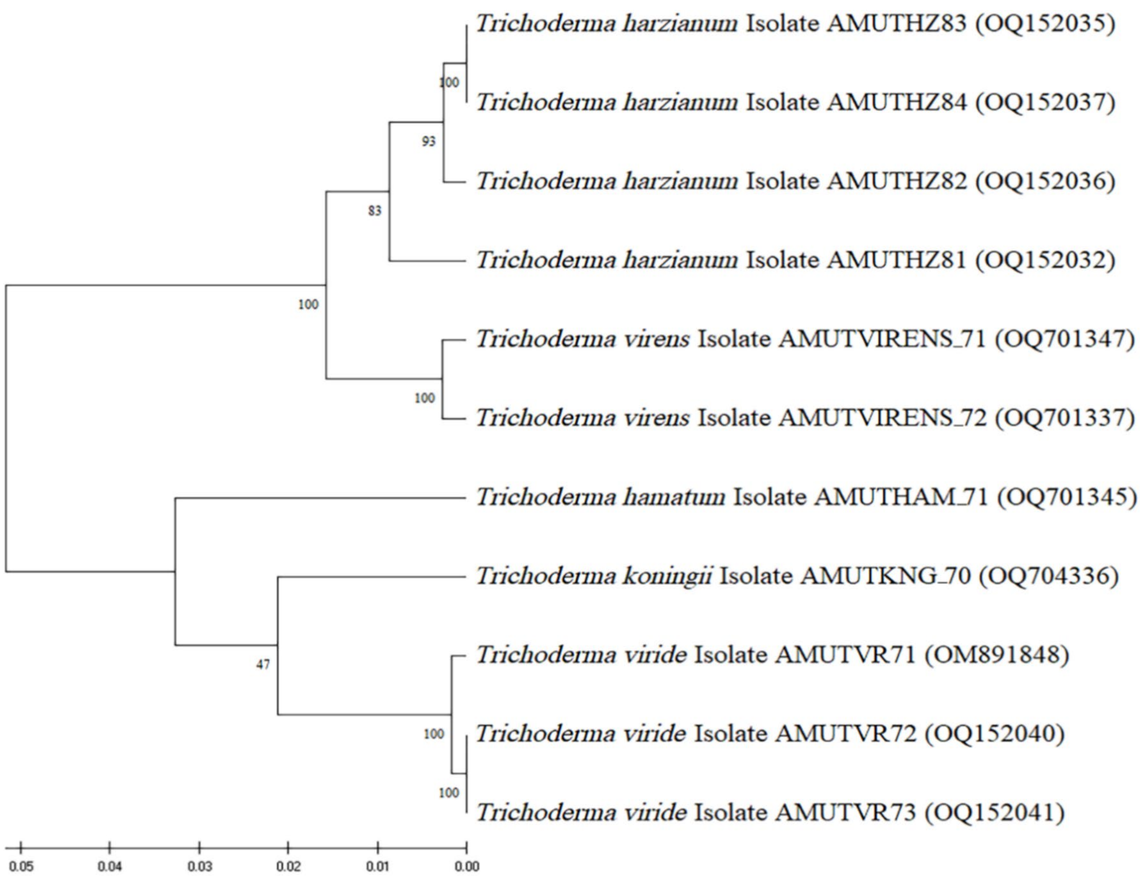

Figure 1. Dendrogram showing the homology similarity search for *Trichoderma harzianum, T. virens, T. viride, T. hamatum* and *T. koningii* isolates.

Trichoderma isolates	Gen Bank accession no
T. harzianum AMUTHZ81	OQ152032
T. harzianum AMUTHZ82	OQ152036
T. harzianum AMUTHZ83	OQ152035
T. harzianum AMUTHZ84	OQ152037
T. viride AMUTVR71	OM891848
T. viride AMUTVR72	OQ152040
T. viride AMUTVR73	OQ152041
T. virens AMUTVIRENS_71	OQ701347
T. virens AMUTVIRENS_72	OQ701337
T. hamatum AMUTHAM_71	OQ701345
T. koningii AMUTKNG_70	OQ704336

Table 1. *Trichoderma* isolates evaluated in the study.

	Soil application				Seed treatment			
	Irrigation				Irrigation			
Treatments	100%	75%	50%	25%	100%	75%	50%	25%
Control	59.5^{cd}	45.8^{fg}	33.2^{hijk}	24.8^{l}	59.5^{bc}	45.8^{ef}	33.2^{ghijkl}	24.8^{m}
T. harzianum AMUTHZ81	70.11^{ab} (17.84)	52.86^{def} (15.42)	37.43^{h} (12.75)	26.27^{kl} (5.94)	68.42^{a} (14.99)	51.2^{de} (11.79)	35.56^{gh} (7.1)	26.1^{jklm} (5.24)
T. harzianum AMUTHZ82	70.72^{ab} (18.86)	54.84^{de} (19.74)	38.98^{gh} (17.43)	27.86^{ijkl} (12.36)	68.12^{a} (14.48)	54.31^{cd} (18.6)	38.54^{fg} (16.1)	27.55^{hijklm} (11.08)
T. harzianum AMUTHZ83	69.3^{ab} (16.47)	54.25^{de} (18.46)	36.8^{h} (10.84)	26.61^{jkl} (7.32)	67.54^{ab} (13.51)	51.2^{de} (11.79)	35.27^{gh} (6.25)	25.84^{klm} (4.19)
T. harzianum AMUTHZ84	73.55^{a} (23.62)	52.30^{def} (14.20)	38.2^{gh} (15.06)	27.76^{ijkl} (11.95)	70.35^{a} (18.25)	52.3^{cde} (14.19)	35.78^{g} (7.78)	26.45^{ijklm} (6.65)
T. viride AMUTVR71	68.6^{ab} (15.29)	51.59^{ef} (12.65)	36.10^{h} (8.75)	26.56^{jkl} (7.10)	67.24^{ab} (13)	51.59^{cde} (12.64)	35.17^{gh} (5.96)	25.34^{lm} (2.17)
T. viride AMUTVR72	68.66^{ab} (15.40)	54.0^{de} (17.95)	38.79^{gh} (16.86)	27.33^{ijkl} (10.22)	70.24^{a} (18.05)	53.75^{cde} (17.35)	34.77^{gh} (4.72)	25.1^{m} (1.2)
T. viride AMUTVR73	71.28^{ab} (19.8)	53.70^{de} (17.26)	38.61^{gh} (16.32)	27.66^{ijkl} (11.54)	69.92^{a} (17.52)	51.12^{de} (11.61)	36.73^{g} (10.65)	26.1^{jklm} (5.24)
T. virens AMUTVIRENS_71	64.8^{bc} (8.90)	51.27^{ef} (11.96)	36.8^{h} (10.84)	26.00^{kl} (5.10)	67.45^{ab} (13.36)	50.68^{de} (10.65)	33.92^{ghij} (2.16)	25.18^{lm} (1.53)
T. virens AMUTVIRENS_72	65.5^{bc} (10.08)	49.71^{ef} (8.55)	35.1^{hi} (5.74)	25.56^{kl} (3.1)	65.11^{ab} (9.42)	49.71^{de} (8.53)	33.74^{ghijk} (1.62)	24.98^{m} (0.72)
T. hamatum AMUTHAM_71	69.7^{ab} (17.14)	52.13^{def} (13.84)	36.37^{h} (9.56)	25.84^{kl} (4.22)	67.84^{a} (14.01)	52.13^{cde} (13.82)	34.2^{ghi} (3.01)	25.11^{m} (1.24)
T. koningii AMUTKNG_70	63.5^{bc} (6.72)	47.26^{ef} (3.20)	34.15^{hij} (2.87)	25.17^{l} (1.5)	62.8^{ab} (5.54)	47.26^{de} (3.18)	33.97^{ghij} (2.31)	24.85^{m} (0.2)
F-value ($P \leq 0.05$)								
Irrigation	2180.36***				2062.31***			
Treatment	11.61***				6.58***			
Irrigation:treatment	1.23*				1.07*			
LSD ($P \leq 0.05$)								
Irrigation	1.08				1.12			
Treatment	1.88				1.94			
Irrigation:treatment	3.76				3.89			

Table 2. Effects of *Trichoderma* spp. against different water stress levels on the plant growth (shoot length (cm)) of rice cultivar Swarna sub-1. Values are mean of 5 replicates. Values in the parenthesis are percent increase over control. Values (means) followed by different alphabets within a column are significantly different at $P \leq 0.05$ according two-factor ANOVA and Tukey HSD test.

of shoot and root i.e., 16.1 and 13.4% (*T. harzianum* AMUTHZ82) and 10.6 and 8.2% (*T. viride* AMUTVR73) under 50% drought condition. Next in effectiveness were *T. viride* AMUTVR72, *T. hamatum* AMUTHAM_71, *T. harzianum* AMUTHZ81 and *T. viride* AMUTVR72 which significantly increased shoot and root length under drought conditions ($P \leq 0.05$; Tables 2, 3). However, under normal irrigation (100% watering) highest promotion in the plant growth of rice was recorded, 21.3–23.6% (soil application) and 18–19.2% (seed treatment with *T. harzianum* AMUTHZ84. Next in effectiveness was *T. viride* AMUTVR73 (19.8 and 17.5%) over untreated control (Tables 2, 3).

Treatments	Soil application				Seed treatment			
	Irrigation				Irrigation			
	100%	75%	50%	25%	100%	75%	50%	25%
Control	$8.5^{abcdefgh}$	6.4^{hij}	$5.5^{ijklmno}$	3.6^{o}	$8.5^{abcdefg}$	6.4^{efghij}	5.5^{hij}	3.6^{j}
T. harzianum AMUTHZ81	9.75^{abcde} (14.82)	7.28^{efghi} (13.82)	6.17^{hijklm} (12.27)	3.90^{klmno} (8.4)	9.55^{abcd} (12.35)	7.14^{defgh} (11.56)	6.03^{fghij} (9.67)	3.87^{ij} (7.5)
T. harzianum AMUTHZ82	9.99^{a} (17.54)	$7.45^{bcdefghi}$ (16.42)	6.27^{hijk} (14.12)	4.0^{jklmno} (11.26)	10.18^{ab} (19.26)	$7.38^{abcdefgh}$ (15.31)	6.24^{fghij} (13.45)	3.95^{ij} (9.72)
T. harzianum AMUTHZ83	9.87^{abc} (16.22)	7.38^{defghi} (15.32)	6.20^{hijklm} (12.75)	3.90^{klmno} (8.4)	10.1^{abc} (18.82)	$7.28^{bcdefgh}$ (13.75)	6.05^{fghij} (10)	3.85^{ij} (6.94)
T. harzianum AMUTHZ84	10.31^{a} (21.36)	7.26^{fghi} (13.56)	$6.13^{hijklmn}$ (11.54)	3.97^{jklmno} (10.45)	10.23^{a} (19.76)	7.18^{defgh} (12.18)	5.92^{ghij} (7.67)	3.81^{ij} (5.88)
T. viride AMUTVR71	9.84^{abcd} (15.80)	7.11^{ghi} (11.17)	6.16^{hijklm} (12.20)	3.85^{klmno} (7.2)	9.75^{abcd} (14.7)	7.0^{defgh} (9.37)	6.02^{fghij} (9.45)	3.81^{ij} (5.88)
T. viride AMUTVR72	9.98^{a} (17.51)	07.34^{efghi} (14.75)	$6.1^{hijklmn}$ (10.70)	3.75^{lmno} (4.22)	9.85^{abc} (15.88)	$7.32^{abcdefgh}$ (14.37)	5.11^{hij} (8.24)	3.9^{ij} (8.33)
T. viride AMUTVR73	10.0^{a} (18.15)	$7.41^{cdefghi}$ (15.84)	6.22^{hijkl} (13.21)	3.93^{jklmno} (9.22)	10.1^{abcd} (18.82)	$7.35^{abcdefgh}$ (14.84)	5.81^{ghij} (5.74)	3.74^{ij} (3.88)
T. virens AMUTVIRENS_71	09.6^{abcdef} (12.94)	07.1^{ghi} (10.62)	$6.10^{hijklmn}$ (10.95)	3.72^{mno} (3.54)	9.56^{abcd} (12.47)	6.95^{defgh} (8.59)	6.07^{fghij} (10.36)	3.69^{j} (2.5)
T. virens AMUTVIRENS_72	9.93^{ab} (16.88)	7.32^{efghi} (14.43)	$6.14^{hijklmn}$ (11.65)	3.79^{klmno} (5.4)	9.76^{abcd} (14.82)	7.24^{cdefgh} (13.12)	5.89^{ghij} (7.23)	3.74^{ij} (3.88)
T. hamatum AMUTHAM_71	9.96^{a} (17.20)	7.21^{fghi} (12.81)	$6.12^{hijklmn}$ (11.34)	3.77^{lmno} (4.8)	9.25^{abcde} (8.82)	7.15^{defgh} (11.71)	5.92^{ghij} (7.63)	3.72^{ij} (3.33)
T. koningii AMUTKNG_70	$9.32^{abcdefg}$ (9.70)	06.8^{hi} (6.25)	$5.8^{ijklmno}$ (5.45)	3.67^{no} (2.1)	8.92^{abcdef} (4.94)	6.62^{efghi} (3.43)	5.71^{ghij} (3.81)	3.65^{j} (1.38)
F-value ($P \leq 0.05$)								
Irrigation	397.73***				286.11***			
Treatment	1.52				1.09			
Irrigation:treatment	0.15				0.26			
LSD ($P \leq 0.05$)								
Irrigation	0.34				0.40			
Treatment	0.60				0.70			
Irrigation:treatment	1.20				1.40			

Table 3. Effects of *Trichoderma* spp. against different water stress levels on the plant growth (root length (cm)) of rice cultivar Swarna sub-1. Values are mean of 5 replicates. Values in the parenthesis are percent increase over control. Values (means) followed by different alphabets within a column are significantly different at $P \leq 0.05$ according two-factor ANOVA and Tukey HSD test.

Plant biomass production

The biomass production of rice plants grown under water stress conditions (75, 50 and 25% water availability of the recommended amount) was adversely affected. The fresh weight of shoot and root decreased by 21–26%, 36–45% and 52–62% at 75, 50, 25% watering, respectively over 100% watering (control). The soil application of *T. harzianum* AMUTHZ82 significantly promoted the plant biomass production under 75 and 50% water availability with a maximum increase 20.6 and 17.6% in shoot fresh weight and 18.5 and 15.3% in root fresh weight respectively, over control ($P \leq 0.05$). Next in effectiveness was *T. viride* AMUTVR73, followed by *T. harzianum* AMUTHZ84 and *T. viride* AMUTVR71 which also significantly promoted the fresh weight of shoot and root ($P \leq 0.05$; Tables 4, 5) over control. Under 25% water availability, *T. harzianum* AMUTHZ82 scored highest increase in fresh weight of shoot and root (13.8 and 11.3% respectively; Tables 4, 5), followed by *T. viride* AMUTVR73 (11.4 and 10.4%), and *T. hamatum* AMUTHAM_71 (8.2 and 8.1%) over control ($P \leq 0.05$).

However, in 100% watered plants, maximum increase in biomass production was recorded with the soil application of *T. harzianum* AMUTHZ84 (22.6% and 21.4% respectively; Tables 4, 5) followed by seed treatment (22.1 and 20.7%). Next in effectiveness were *T. viride* AMUTVR73 (20.4 and 20.9%), *T. harzianum* AMUTHZ83 (19.3 and 19.3%) and *T. viride* AMUTVR71 (11.8 and 18.4%) over respective control. Other strains viz., *T. koningii* AMUTKNG_70 and *T. virens* AMUTVIRENS_71 did not significantly influence the biomass production of rice over respective control ($P \leq 0.05$; Tables 4, 5). Overall, the soil application of *Trichoderma* AMUTHZ82 isolates induced 7–11% greater enhancement in the biomass production of rice in comparison to seed treatment.

Grain yield

The water stress caused significant decline (12–51%) in per plant grain yield production of rice cv. Swarna Sub-1 over normal irrigated control (Table 6). However, soil application of *Trichoderma* spp. improved the grain yield in both normal and water stress conditions. In normal irrigated condition, maximum increase in production of grain yield was recorded with the treatment of *T. harzianum* AMUTHZ84 (24.68%), followed by *T. viride* AMUTVR73 and *T. harzianum* AMUTHZ82 (22.08 and 20.13%, respectively; Table 6). Under 75 and 50% of water availability, *T. harzianum* AMUTHZ82 was found superior among all isolates in increasing grain yield by22.79 and 19.63%, respectively, over control (Table 6). Next in effectiveness were *T. viride* AMUTVR73, *T. harzianum* AMUTHZ84 and *T. hamatum* AMUTHM_71 which significantly increased grain yield of rice under drought conditions over control. Under 25% water availability of recommended amount, none of the isolates of *Trichoderma* were found effective in significantly enhancing the grain yield of rice plants over control. Overall, the soil application of *Trichoderma* AMUTHZ82 isolates induced 10–22% greater grain yield production of rice in comparison to seed treatment (Table 6).

Treatments	Soil application				Seed treatment			
	Irrigation				Irrigation			
	100%	75%	50%	25%	100%	75%	50%	25%
Control	52.4^{cd}	38.4^{efg}	28.7^{hij}	19.6^{k}	52.4^{cd}	38.4^{efghi}	28.7^{jklm}	19.6^{n}
T. harzianum AMUTHZ81	58.4^{abc} (11.45)	44.5^{de} (15.88)	32.26^{fgh} (12.42)	21.0^{jk} (7.23)	59.85^{abc} (14.21)	43.72^{e} (13.85)	32.15^{hij} (12.02)	20.95^{mn} (6.88)
T. harzianum AMUTHZ82	61.46^{ab} (17.30)	46.32^{de} (20.65)	33.75^{fgh} (17.60)	22.31^{ijk} (13.85)	60.43^{abc} (15.34)	46.24^{de} (20.41)	33.46^{fghij} (16.58)	22.25^{klmn} (13.52)
T. harzianum AMUTHZ83	62.51^{ab} (19.30)	45.2^{de} (17.70)	32.95^{fgh} (14.81)	22.1^{jk} (12.55)	61.59^{abc} (17.53)	44.51^{de} (15.91)	32.76^{hij} (14.14)	22.0^{klmn} (12.24)
T. harzianum AMUTHZ84	64.24^{a} (22.61)	45.47^{de} (18.42)	33.22^{fgh} (15.78)	21.77^{jk} (11.10)	63.95^{a} (22.04)	44.34^{de} (15.46)	32.95^{hij} (14.8)	21.54^{klmn} (9.89)
T. viride AMUTVR71	58.6^{abc} (11.83)	44.64^{de} (16.27)	33.2^{fgh} (15.67)	21.70^{jk} (10.75)	56.87^{abc} (8.53)	43.76^{e} (13.95)	32.49^{hij} (13.2)	21.43^{klmn} (9.33)
T. viride AMUTVR72	57.9^{abc} (10.58)	45.66^{de} (18.92)	32.7^{fgh} (13.93)	21.15^{jk} (7.95)	57.54^{abc} (9.8)	44.21^{de} (15.13)	32.68^{hij} (13.86)	21.1^{lmn} (7.65)
T. viride AMUTVR73	63.10^{ab} (20.43)	45.92^{de} (19.55)	33.38^{fgh} (16.32)	21.84^{jk} (11.43)	62.31^{ab} (18.91)	45.26^{de} (17.86)	33.28^{ghij} (15.95)	21.75^{klmn} (10.96)
T. virens AMUTVIRENS_71	56.6^{abc} (8.19)	42.32^{e} (7.63)	30.37^{ghi} (5.83)	20.23^{k} (3.24)	55.74^{abc} (6.37)	41.82^{efg} (8.9)	29.78^{jk} (3.76)	20.15^{n} (2.8)
T. virens AMUTVIRENS_72	57.5^{abc} (9.77)	42.7^{e} (11.19)	31.2^{gh} (8.71)	20.25^{k} (3.36)	56.34^{abc} (7.51)	41.84^{ef} (8.958)	30.75^{ij} (7.14)	20.17^{n} (2.9)
T. hamatum AMUTHAM_71	59.6^{abc} (13.74)	43.1^{e} (12.23)	31.54^{gh} (9.92)	21.20^{jk} (8.21)	57.68^{abc} (10.07)	42.67^{e} (11.11)	31.1^{ij} (8.36)	20.85^{mn} (6.37)
T. koningii AMUTKNG_70	55.3^{bc} (5.67)	40.26^{ef} (4.85)	29.2^{hij} (1.74)	19.89^{k} (1.54)	55.12^{bc} (5.19)	40.26^{efgh} (4.84)	29.43^{jkl} (2.54)	19.85^{n} (1.27)
F-value ($P \le 0.05$)								
Irrigation	1581.51***				1452.43***			
Treatment	8.29***				7.02***			
Irrigation:treatment	0.80				0.72			
LSD ($P \le 0.05$)								
Irrigation	1.14				1.17			
Treatment	1.98				2.04			
Irrigation:treatment	3.97				4.08			

Table 4. Effects of *Trichoderma* spp. against different water stress levels on the plant biomass (Shoot fresh weight (gm)) of rice cultivar Swarna sub-1. Values are mean of 5 replicates. Values in the parenthesis are percent increase over control. Values (means) followed by different alphabets within a column are significantly different at $P \le 0.05$ according two-factor ANOVA and Tukey HSD test.

Photosynthesis and transpiration rate

Rice plants grown under drought conditions experienced decline in the photosynthesis rate and transpiration rate (Figs. 2, 3). This resulted in reduced plant growth, and quality biomass production. The photosynthesis rate was found maximum in the rice plants grown under normal condition (100% water availability) with the soil application of *T. harzianum* AMUTHZ82 (7.12 µmol m^{-2} s^{-1}), followed by *T. viride* AMUTVR72, *T. harzianum* AMUTHZ84, *T. harzianum* AMUTHZ83 and *T. hamatum* AMUTHAM_71. Lowest photosynthesis rate was recorded under 25% of water availability in comparison to 100% water availability. Soil application with *T. harzianum* AMUTHZ82 significantly enhanced the photosynthesis rate of rice plants growing under 75% (37% increase), 50% (19% increase) and 25% water availabilities (9% increase) over respective control. The seed treatment with *Trichoderma* spp. also minimized the negative effect of water stress on photosynthesis, but less than the soil application.

The transpiration rate was also influenced under low water availability with the treatment of *Trichoderma* spp. The plants treated with *T. harzianum* AMUTHZ82 showed minimum transpiration rate (2.36 µmol m^{-2} s^{-1}), followed by *T. viride* AMUTVR72, *T. hamatum* AMUTHAM_71 and *T. harzianum* AMUTHZ84 under normal irrigated condition. The reduction in transpiration rate was observed lesser under 50% and 25% drought condition with the treatment of *Trichoderma* spp. over normal irrigated plants. Whereas, maximum transpiration rate was observed in untreated plant receiving recommended level of irrigation (Fig. 2, 3).

Leaf pigments

Total chlorophyll content (Chlorophyll A and B) in the leaves of rice plants under water stress conditions (75, 50 and 25% water availability of recommended amount) was recorded with a 20, 37 and 49% decline, respectively over control (Fig. 4). Under regular irrigation (100% watering), soil application of *T. harzianum* AMUTHZ84 resulted a maximum increase (21.3%) in chlorophyll content, followed by *T. viride* AMUTVR73 (17%) in the leaves of rice plants. Whereas, under 75 and 50% watering regime, *T. harzianum* AMUTHZ82 increased the total chlorophyll content (16.6 and 13.1%) over respective control (Figs. 4, 5). *T. harzianum* AMUTHZ83 and *T. viride* AMUTVR73 were found next in increasing chlorophyll content under 25% and 50% water stress conditions.

Treatments	Soil application				Seed treatment			
	Irrigation				Irrigation			
	100%	75%	50%	25%	100%	75%	50%	25%
Control	12.3^{bcdef}	9.6^{ghij}	7.8^{jklmno}	5.8^{o}	12.3^{abcde}	9.6^{fghi}	7.8^{ijklmno}	5.8^{o}
T. harzianum AMUTHZ81	14.5^{ab} (17.88)	10.8^{defghi} (12.5)	8.82^{ghijklm} (13.20)	6.29^{mno} (8.45)	14.46^{b} (17.56)	10.92^{cdefg} (13.75)	8.79^{fghijklm} (12.69)	6.24^{lmno} (7.58)
T. harzianum AMUTHZ82	14.54^{ab} (18.26)	11.37^{cdefg} (18.53)	8.99^{ghijk} (15.32)	6.45^{klmno} (11.36)	14.67^{a} (19.26)	11.23^{bcdef} (16.97)	8.97^{fghij} (15)	6.44^{jklmno} (11.03)
T. harzianum AMUTHZ83	14.82^{ab} (20.54)	11.1^{cdefgh} (15.53)	8.91^{ghijkl} (14.25)	6.30^{mno} (8.63)	14.75^{a} (19.91)	11.0^{bcdefg} (14.58)	8.75^{fghijklm} (12.17)	6.29^{klmno} (8.44)
T. harzianum AMUTHZ84	14.93^{a} (21.43)	11.25^{cdefg} (17.25)	8.93^{ghijkl} (14.56)	6.35^{lmno} (9.55)	14.85^{a} (20.73)	11.12^{bcdefg} (15.83)	8.87^{fghijkl} (13.71)	6.27^{lmno} (8.1)
T. viride AMUTVR71	14.57^{ab} (18.46)	11.15^{cdefgh} (16.22)	8.78^{ghijklm} (12.67)	6.37^{lmno} (9.92)	14.52^{b} (18.04)	10.96^{cdefg} (14.16)	8.73^{fghijklm} (11.92)	6.34^{jklmno} (9.311)
T. viride AMUTVR72	13.7^{abc} (11.38)	10.3^{fghij} (7.29)	8.82^{ghijklm} (13.11)	6.23^{mno} (7.46)	13.65^{efghi} (10.97)	10.26^{efghi} (6.87)	8.76^{fghijklm} (12.3)	6.21^{lmno} (7.06)
T. viride AMUTVR73	14.87^{ab} (20.92)	11.31^{cdefg} (17.86)	8.99^{ghijk} (15.34)	6.40^{klmno} (10.40)	14.82^{a} (20.48)	10.18^{efghi} (6.04)	8.95^{fghijk} (14.74)	6.38^{jklmno} (10)
T. virens AMUTVIRENS_71	13.26^{abcde} (7.87)	9.93^{fghij} (3.45	7.9^{jklmno} (2.5)	5.91^{o} (1.95)	13.2^{abcd} (7.31)	9.76^{efghi} (1.66)	7.87^{ijklmno} (0.89)	5.89^{no} (1.55)
T. virens AMUTVIRENS_72	13.4^{abcd} (8.94)	10.35^{fghij} (7.85)	8.24^{ijklmno} (5.76)	6.17^{no} (6.47)	13.36^{abc} (8.61)	10.21^{efghi} (6.35)	8.17^{hijklmno} (4.74)	6.14^{mno} (5.86)
T. hamatum AMUTHAM_71	14.2^{ab} (15.44)	10.71^{efghi} (11.62)	8.6^{hijklmn} (10.25)	6.27^{mno} (8.25)	14.12^{b} (14.79)	10.54^{defgh} (9.79)	8.54^{ghijklmn} (9.48)	6.23^{lmno} (7.41)
T. koningii AMUTKNG_70	13.3^{abcde} (6.82)	09.8^{fghij} (2.08)	7.9^{jklmno} (1.87)	5.87^{o} (1.35)	13.0^{abcd} (5.69)	10.1^{efghi} (5.2)	8.0^{hijklmno} (2.56)	5.86^{o} (1.03)
F-value ($P \leq 0.05$)								
Irrigation	657.30***				620.39***			
Treatment	5.42***				4.51***			
Irrigation:treatment	0.41				0.48			
LSD ($P \leq 0.05$)								
Irrigation	0.36				0.37			
Treatment	0.63				0.64			
Irrigation:treatment	1.26				1.28			

Table 5. Effects of *Trichoderma* spp. against different water stress levels on the plant biomass (root fresh weight (gm)) of rice cultivar Swarna sub-1. Values are mean of 5 replicates. Values in the parenthesis are percent increase over control. Values (means) followed by different alphabets within a column are significantly different at $P \leq 0.05$ according two-factor ANOVA and Tukey HSD test.

The plants treated through seed treatment of *Trichoderma* isolates under water stress had significantly greater content of leaf pigment, but less than soil application. The plants receiving 25% water of the recommended amount recorded with 2–7% increase in total chlorophyll content with the soil application or seed treatment with *Trichoderma* isolates (Figs. 4, 5).

Discussion

The rice cv. Swarna Sub-1 was found sensitive to drought (water stress), and exhibited significant reduction in the plant growth, biomass production, leaf pigments, photosynthesis etc. grown under water stress conditions (75, 50 and 25% water of the recommended amount) in comparison to plants received normal irrigation (100% water availability of the recommended level). Adequate supply of water to a plant is essential for carrying out various physiological, biological and biochemical activities of the plant[28,29]. A decrease in the water supply influences the physiological activities especially the photosynthesis[30,31], and subsequently the plant growth and development[32], which directly affect the biomass production[33,34] as observed in the present study.

The soil application of *Trichoderma* spp. especially *T. harzianum* AMUTHZ82 and *T. viride* AMUTVR73 subsided the negative effect of water stress on rice plants, and promoted the shoot and root growth, and biomass production of rice cv. Swarna Sub-1. *Trichoderma* spp. are universally occurring beneficial microbes in varied kind of soils[13], and are generally known to suppress plant pathogenic fungi[17,28], by parasitizing them or by producing metabolites, toxins, enzymes, etc. toxic to pathogens[28,35]. *Trichoderma* spp. also solubilize minerals present in soil, and produce hormones[36,37], which may ultimately lead to plant growth promotion[14,38,39]. Some strains of *Trichoderma* spp. form endophytic relationship with roots, and develop symbiotic association with plants[13,40]. With such association, plants are able to efficiently use the water and nutrients in soil[41], and are able to survive under various stress conditions such as nutrition deficiency, water stress, etc.[42,43].

Khan et al.[44] conducted a study in which the application of *T. harzianum* significantly enhanced plant height, root length, and shoot biomass of rice cv. Swarna Sub-1, under water-deficient conditions. Similarly, Haque et al.[45] demonstrated that *T. harzianum* treated rice plants exhibited improved water-use efficiency, enhanced

Treatments	Soil application				Seed treatment			
	Irrigation				Irrigation			
	100%	75%	50%	25%	100%	75%	50%	25%
Control	15.4^{b-j}	13.6^{g-o}	10.7^{o-v}	7.4^{v}	15.4^{b-e}	13.6^{d-j}	10.7^{j-p}	7.4^{p}
T. harzianum AMUTHZ81	18.3^{a-d} (18.83)	15.2^{c-k} (11.76)	11.8^{k-p} (10.28)	8.0^{q-v} (8.11)	17.9^{ab} (16.23)	15.5^{b-e} (13.97)	11.5^{g-k} (7.48)	7.9^{m-p} (6.76)
T. harzianum AMUTHZ82	18.5^{a-c} (20.13)	16.3^{a-h} (19.85)	12.1^{j-o} (13.08)	8.2^{q-v} (10.81)	17.8^{ab} (15.58)	15.6^{a-e} (14.71)	11.8^{f-j} (10.28)	8.1^{l-p} (9.46)
T. harzianum AMUTHZ83	17.6^{a-f} (14.29)	15.4^{b-j} (13.24)	11.8^{k-p} (10.28)	7.8^{s-v} (5.41)	17.4^{a-c} (12.99)	15.3^{b-e} (12.5)	11.5^{g-k} (7.48)	7.7^{n-p} (4.05)
T. harzianum AMUTHZ84	19.2^{a} (24.68)	16.7^{a-g} (22.79)	12.8^{h-o} (19.63)	8.5^{p-v} (14.86)	18.9^{a} (22.73)	16.4^{a-d} (20.59)	12.5^{e-j} (16.82)	8.4^{k-p} (13.51)
T. viride AMUTVR71	16.8^{a-g} (9.09)	14.8^{d-l} (8.82)	11.5^{l-q} (7.48)	7.9^{r-v} (6.76)	16.4^{a-d} (6.49)	14.4^{c-g} (5.88)	11.2^{g-n} (4.67)	7.7^{n-p} (4.05)
T. viride AMUTVR72	16.6^{a-g} (7.79)	14.6^{e-m} (7.35)	11.4^{l-r} (6.54)	7.9^{r-v} (6.76)	16.2^{a-d} (5.19)	14.3^{c-h} (5.15)	11.1^{g-n} (3.74)	7.6^{op} (2.7)
T. viride AMUTVR73	18.8^{ab} (22.08)	15.7^{a-i} (15.44)	12.2^{i-o} (14.02)	8.1^{q-v} (9.46)	18.3^{ab} (18.83)	15.7^{a-e} (15.44)	11.9f.$^{-j}$ (11.21)	8.0^{l-p} (8.11)
T. virens AMUTVI-RENS_71	16.4^{a-g} (6.49)	14.3^{e-n} (5.15)	11.0^{n-u} (2.8)	7.6^{u-v} (2.7)	16.3^{a-d} (5.84)	14.2^{c-i} (4.41)	11.0^{h-n} (2.8)	7.5^{p} (1.35)
T. virens AMUTVI-RENS_72	16.3^{a-h} (5.84)	14.2^{e-o} (4.41)	11.2^{m-t} (4.67)	7.7^{t-v} (4.05)	16.2^{a-d} (5.19)	14.1^{c-i} (3.68)	11.1^{g-n} (3.74)	7.6^{op} (2.7)
T. hamatum AMUTHAM_71	17.7^{a-e} (14.94)	14.7^{e-m} (8.09)	11.3^{l-s} (5.61)	7.6^{u-v} (2.7)	17.2^{a-c} (11.69)	15^{b-f} (10.29)	11.3^{g-l} (5.61)	7.7^{n-p} (4.05)
T. koningii AMUT-KNG_70	16.2^{a-h} (5.19)	14.1^{f-o} (3.68)	10.9^{n-v} (1.87)	7.5^{u-v} (1.35)	16.1^{a-d} (4.55)	14.1^{c-i} (3.68)	10.9^{i-o} (1.87)	7.5^{p} (1.35)
F-value ($P \leq 0.05$)								
Irrigation	557.22***				580.71***			
Treatment	6.08***				5.26***			
Irrigation:treatment	0.49				0.39			
LSD ($P \leq 0.05$)								
Irrigation	0.49				0.47			
Treatment	0.84				0.76			
Irrigation:treatment	1.69				1.54			

Table 6. Effects of *Trichoderma* spp. against different water stress levels on the grain yield (gm/plant) of rice cultivar Swarna sub-1. Values are mean of 5 replicates. Values in the parenthesis are percent increase over control. Values (means) followed by different alphabets within a column are significantly different at $P \leq 0.05$ according two-factor ANOVA and Tukey HSD test.

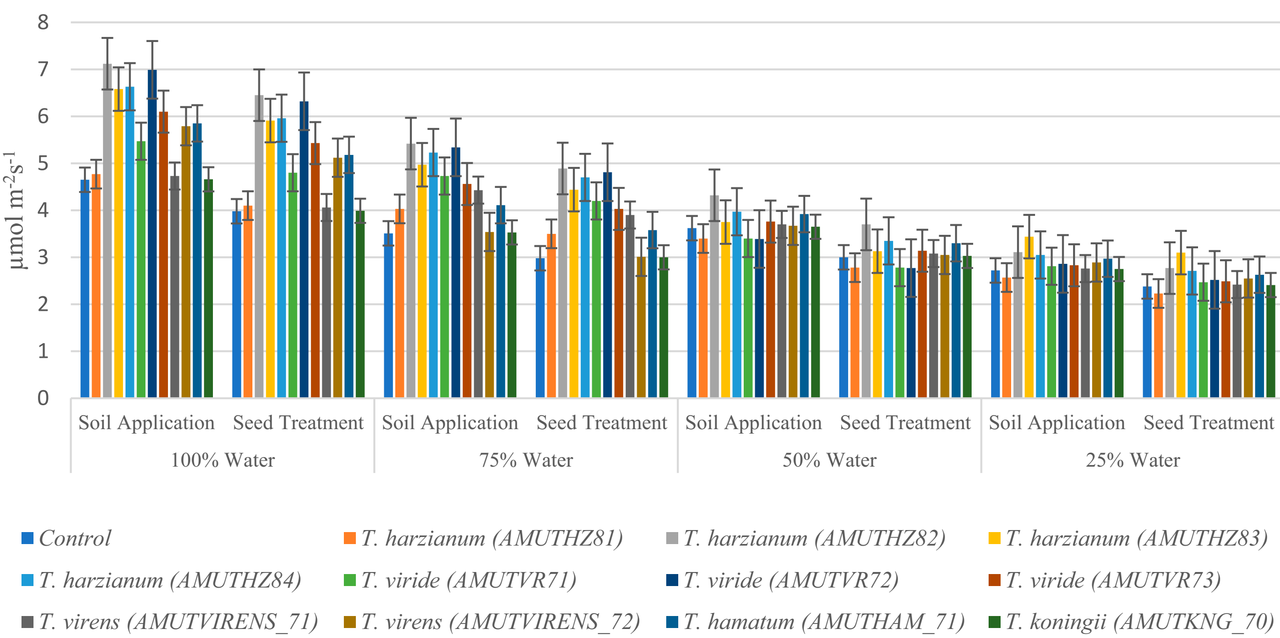

Figure 2. The photosynthesis rate in the leaves of rice plants under different water stress levels. Error bars show standard error.

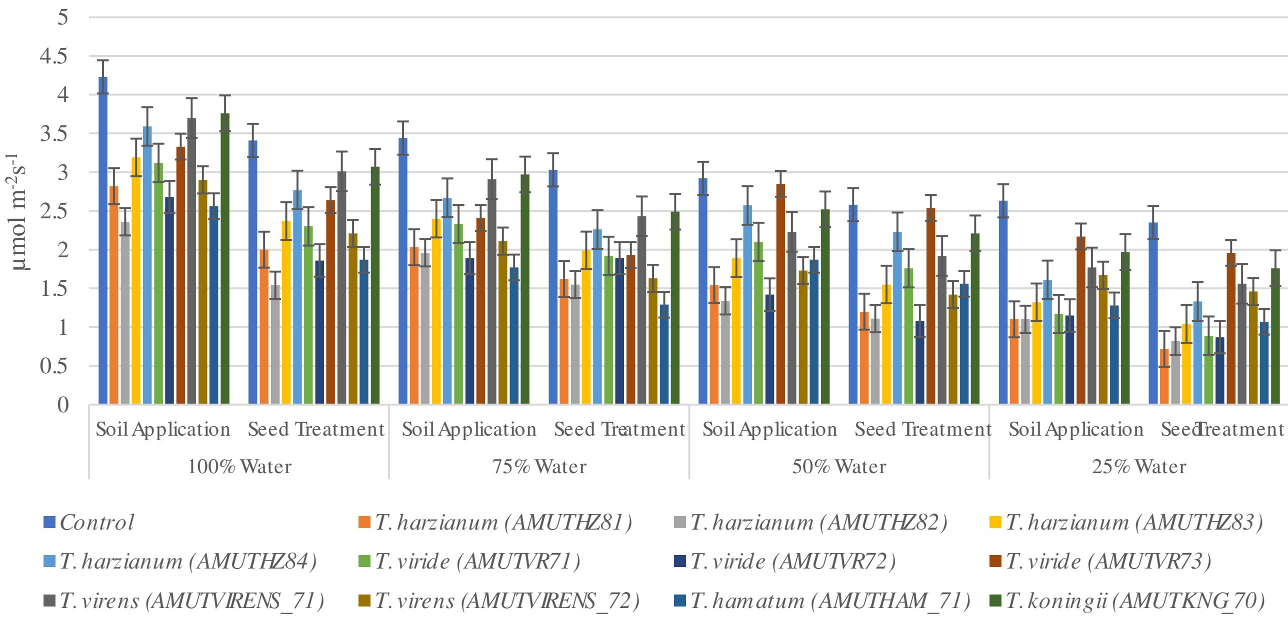

Figure 3. The transpiration rate in the leaves of rice plants under different water stress levels. Error bars show standard error.

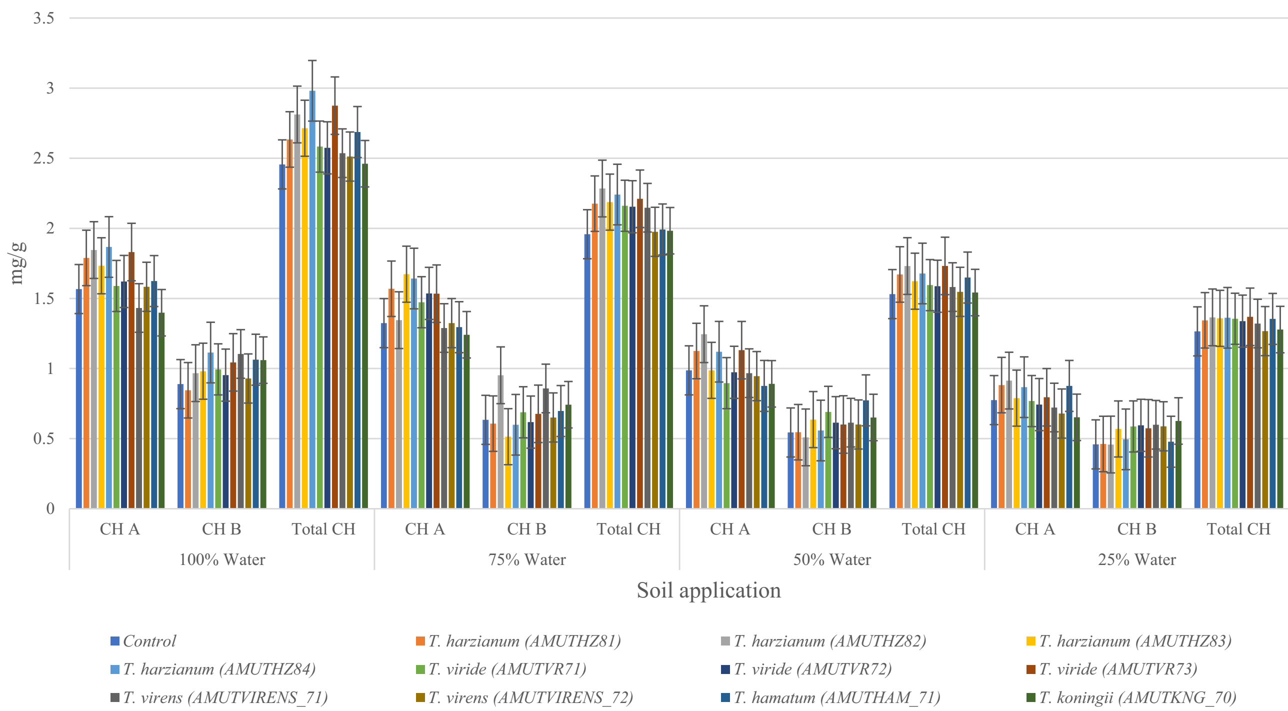

Figure 4. Effects of soil application of *Trichoderma* isolates on the total chlorophyll (chlorophyll A and B) content in the leaves of rice plants under different water stress levels. Error bars show standard error. Where CH = Chlorophyll.

photosynthetic activity, and increased grain yield compared to non-treated plants during drought stress. *Trichoderma* spp. enhance plant health and stimulate the synthesis of plant growth-promoting substances such as auxins and cytokinins[28], which play crucial roles in root development, nutrient uptake, and overall plant growth[46,47]. Additionally, *T. harzianum* triggers the expression of stress-responsive genes in rice, activating defence mechanisms and antioxidant systems that counteract the detrimental effects of water stress. The present study has demonstrated that soil application of *T. harzianum* AMUTHZ82 or *T. viride* AMUTVR73 may efficiently subside the impact of water stress at least up to 50% water availability levels and may enhance the plant growth and biomass production by 17–21%.

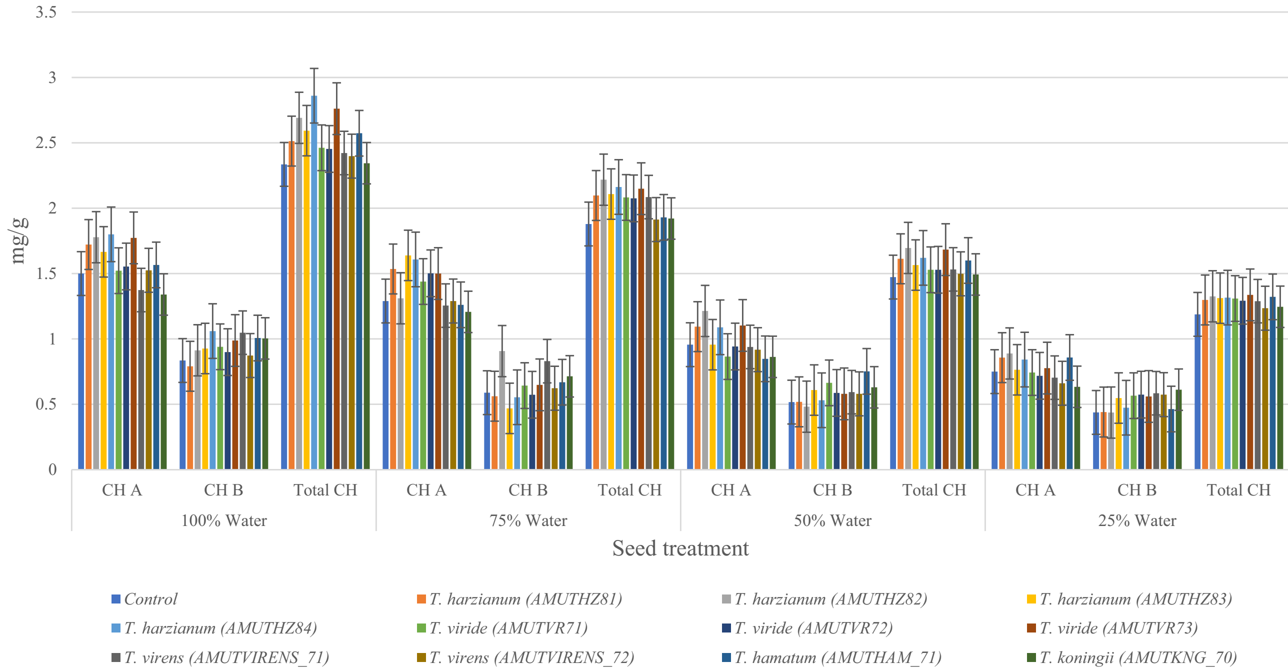

Figure 5. Effects of seed treatment of *Trichoderma* isolates on the total chlorophyll (chlorophyll A and B) content in the leaves of rice plants under different water stress levels. Error bars show standard error. Where CH = Chlorophyll.

Conclusion

The rice cv. Swarna Sub-1 was found sensitive to drought (water stress), and exhibited significant reductions in the plant growth, biomass production, leaf pigments, photosynthesis etc. grown under water stress conditions (75, 50 and 25% water of the recommended amount) in comparison to plants received normal irrigation (100% water availability of the recommended level). The soil application of *Trichoderma* spp. especially *T. harzianum* AMUTHZ82 and *T. viride* AMUTVR73 subsided the negative effect of water stress on rice plants, and promoted the shoot and root growth and biomass production of rice. The present study has demonstrated that soil application of *T. harzianum* AMUTHZ82 or *T. viride* AMUTVR73 may efficiently reduce the impact of water stress at least up to 50% water availability levels and may enhance the plant growth and biomass production by 17–21%.

Materials and methods

Seeds of rice cv. Swarna Sub-1 were procured from Regional Centre, International Rice Research Institute, New Delhi, India.

Isolation and characterization of *Trichoderma* species

The local isolates of different *Trichoderma* spp. were isolated from rice fields using the serial dilution method on selective medium[21]. The corresponding GPS locations (latitude and longitude) of sampling sites were given in Table 7. The plates after inoculation were kept in an incubator at 25 ± 2 °C for 10 days. However, the fungus almost fully colonized the medium in 7 days. After incubation the *Trichoderma* colonies were pure cultured using the same culture medium and conditions. Thereafter, morphological characters of the *Trichoderma* isolates were studied to identify the species, followed by molecular characterization to confirm the identity[48]. The DNA of *Trichoderma* species was sequenced by Macrogen® Incorporation, South Korea, and the homology search was carried out using BLAST on the 18s rRNA-ITS region gene sequences to find and obtain the closest neighbour sequences from the NCBI database[49]. The MEGA-X software was used to access the Clustal-W alignment tool, and used to align all of the sequences. Simple comparison with the database of known species/isolates of *Trichoderma harzianum*, *T. viride*, *T. virens*, *T. hamatum* and *T. konigii* allowed the identification of the species. The UPGMA algorithm included in MEGA-X was used to create the phylogenetic tree (Fig. 1). The sequences have been deposited to the NCBI database and GenBank accession numbers listed in Table 1.

Mass multiplication of *Trichoderma* spp.

Eleven isolates of *Trichoderma* spp. as enlisted in Table 1 were mass cultured on sorghum grains in conical flasks. The seeds were soaked for 12 h. in distilled water containing sucrose (5%) and chloramphenicol (0.0003%). The seeds were kept in 500 ml capacity conical flasks and sterilized at 15 kg/cm^2 steam pressure at 121 °C for 20 min. Next day the flasks were inoculated with the fungus, and incubated in an incubator for 10 days at 25 ± 2 °C. The sorghum seeds colonized by *Trichoderma* spp. were crushed with distilled water in an electrical grinder. The fungal suspension containing 10 g *Trichoderma* colonized seeds was applied to the soil (1 kg) in each pot a day

Districts	Block	Location 1	Location 2	Location 3	Location 4	Location 5	Location 6	Location 7	Location 8	Location 9	Location10
Aligarh	Atrauli	28.03695863076469, 78.30254016347322	28.055672416186297, 78.28331784635671	28.052561346042026, 78.31009900300079	28.04460068082621, 78.32760839868698	28.029082896747052, 78.313957277128	28.054157225072437, 78.28640700145266	28.049764915869783, 78.26932674481777	28.059157218012693, 78.27653671906526	28.070292042363942, 78.26984158513682	28.06286845602028, 78.25919777429573
	Iglas	27.747731226254334, 77.93043322144926	27.779654471801553, 77.92255452685865	27.73558827275805, 77.88165634196875	27.735850581217143, 77.97061273634841	27.70180522519336, 77.96885917921088	27.697828388469656, 77.9959855208573	27.678715142461076, 77.95235674744006	27.70791255314217, 77.92662799452128	27.749224444028652, 77.97337636758421	27.683909962914125, 77.90121032949111
	Akrabad	27.813607706349465, 78.26983809256137	27.81990969170233, 78.26546123223295	27.825452675310746, 78.25464523972524	27.822109807790714, 78.2779953599337	27.80692217533616, 78.29095319708738	27.7981182961571, 78.27833244430512	27.802143694627663, 78.2694927194833	27.79979042108867, 78.26743244936789	27.80571250745651, 78.26503002998167	27.80047451587887, 78.25884978434725
	Lodha	27.90652861925797, 77.98752636539164	27.912894295187623, 77.99577412860859	27.92444126559606, 77.96934068848012	27.91322153171977, 77.95731403612436	27.88165999729069, 77.97068290782096	27.934452259318665, 77.9720960133932	27.91049878516397, 77.93842787467884	27.905027788726994, 77.96108576664939	27.901083189556488, 77.961769886236	27.899249595299068, 77.98339952046024
Kasganj	Kasganj	27.819492467793175, 78.67302451855105	27.82321193198936, 78.67646118752195	27.812200919242866, 78.6798896183754	27.802028860483418, 78.67387456841304	27.78677403802595, 78.65404106204717	27.785180812237286, 78.64889130075757	27.798014509724933, 78.6247764811477	27.812135834438404, 78.62211575420183	27.814944848274294, 78.62838198768816	27.823524222464926, 78.62237317425802
	Sidhpura	27.638294978357578, 78.85612946314109	27.633276245405476, 78.85647289880698	27.630956848296027, 78.85188102783995	27.628409613537254, 78.85720251830962	27.62468327454444, 78.86694375932123	27.62411246153735, 78.87230790416058	27.628484174958807, 78.8781021017459	27.63357870296128, 78.88054941312585	27.640613602652717, 78.8732542700429	27.64221081842372, 78.86939163098842
	Patiyali	27.679558919828985, 78.99285373965743	27.68100084266455, 79.0058139746475	27.68350824013607, 79.00907623676221	27.692551860609715, 79.01268414539564	27.695972881946737, 79.00899395426153	27.698709881907224, 79.00453084288067	27.698938937028053, 78.99766347049535	27.699623796524854, 78.98950869307097	27.698255929707464, 78.98684753245415	27.70053625856723, 78.97809203542079
	Sahawar	27.799296951670513, 78.84291339592335	27.801460837191772, 78.84424445879885	27.795803485226433, 78.84690429163383	27.79356375815594, 78.84535864484992	27.787907206884935, 78.84329745871887	27.78904789212206, 78.82098190898085	27.78870610606701, 78.81686219204933	27.790794035104554, 78.814287208547	27.808334031200413, 78.80123911653457	27.81266249263256, 78.81248467624268
Bulandshahar	Siana	28.614325072498566, 78.05794494528784	28.614625124929862, 78.06506840343808	28.62125331180911, 78.07262313810085	28.62426510959073, 78.07914776670899	28.629613889145194, 78.08069499820124	28.643478645709813, 78.07348902254986	28.646946674713664, 78.06044059345768	28.646269572308125, 78.05108291382928	28.638284041559114, 78.03940770243717	28.630598887763366, 78.02533104680576
	Khurja	28.267449968324073, 77.83599503847711	28.268583560238742, 77.82337562467534	28.258981012585412, 77.82080178467272	28.25013500325033, 77.8213181319003	28.240533606760362, 77.83015927377308	28.233502087835653, 77.8318760087064	28.232594486625946, 77.84620750589394	28.25897770455203, 77.87170471796323	28.26736914811849, 77.87746012270719	28.270320962671097, 77.86338093339175
	Pahasu	28.181081742836543, 78.06237542619387	28.18217927570946, 78.05525095990536	28.18308729735486, 78.04610924588684	28.180968606926214, 78.04173164881952	28.17283516054329, 78.04525128108784	28.169922191465634, 78.04486516373814	28.16629034299277, 78.055722128926	28.170184975635177, 78.07263108909031	28.174233504645855, 78.0692842554637	28.185809372375267, 78.07426583844072
	Shikarpur	28.26655170460858, 78.02358516097217	28.254009051961113, 78.0081310508713	28.27154751816416, 78.003673937408	28.274270517851264, 77.99612153407742	28.282888026051378, 77.99457870541045	28.293168873164753, 77.99097579333454	28.30057329665739, 78.00814851848568	28.30087017848091, 78.02549252925847	28.283632946550235, 78.02994688368703	28.267910044029424, 78.02890775006931
Hathras	Sadabad	27.43117719949259, 78.01575499512114	27.428662867562768, 78.02528148419765	27.422035195561573, 78.02734055828398	27.424469963660613, 78.04407676869873	27.428199678930778, 78.05515033338165	27.438026536010618, 78.05652742433068	27.443283505571458, 78.05472668593782	27.45219507220861, 78.0606545569874	27.457607838586156, 78.04451549296277	27.458143204922678, 78.02906216117837
	Sasni	27.701936269908032, 78.06204525717267	27.709383604001744, 78.05741028022854	27.718350899281106, 78.06170237696999	27.720478112817865, 78.07406446473016	27.71986802208765, 78.0894315940102	27.717890323067138, 78.0976729402343	27.710974718739173, 78.09689756401632	27.70588188110301, 78.10075879588067	27.700942363012295, 78.09981259361238	27.693877058426942, 78.09122649497873
	Sikandrao	27.705644531830817, 78.35472767895105	27.698043519563658, 78.34262456026583	27.686643833376596, 78.36133695127508	27.676382502624577, 78.37352336735783	27.67584887808405, 78.38296402619284	27.677137978839013, 78.3948088805951	27.692416032139388, 78.39618670664342	27.69902902934906, 78.38841930929617	27.696595276919002, 78.40236827372733	27.69986272845902, 78.40683340937127
	Hathars	27.623291044976867, 78.03342680797638	27.615684960122383, 78.00749787709228	27.598492860027697, 78.00852873034188	27.597882428833593, 77.98878615559806	27.563652445979468, 78.01110420669859	27.57460620381112, 78.02517838531081	27.559381956243158, 78.06276263655334	27.575045235855303, 78.08096577249226	27.598167770814044, 78.08990926414668	27.586594661141426, 78.10501181543427
Mathura	Baldeo	27.430787419032132, 77.8487867314326	27.417756835261013, 77.85376106400997	27.410366489359138, 77.85204155422912	27.405567950299154, 77.84603160679194	27.40297913750937, 77.83856385475559	27.40221892702453, 77.82852229121934	27.403438891945818, 77.81899618728113	27.411591516439458, 77.81419033901824	27.42210510525362, 77.82655087668996	27.423321934790536, 77.84165889995184
	Raya	27.57054710431241, 77.78436636473991	27.567578278650384, 77.79277849831146	27.556847059593615, 77.80144559088953	27.550529835811776, 77.80607888897637	27.546270270471606, 77.7989534469832	27.54497796216987, 77.79277349587606	27.543762044734045, 77.78204523186115	27.55433975921481, 77.77397846678792	27.56126417581887, 77.77629636079664	27.56841702659699, 77.77664027588997
	Chhata	27.74189196302714, 77.49902417517832	27.74166350712339, 77.50408924877935	27.73611449856359, 77.51816715409836	27.72805879467711, 77.52468877501038	27.721143810462205, 77.52674643125522	27.71970661778882, 77.48649001526377	27.727380418303756, 77.4871768142597	27.706029249550514, 77.50125076355845	27.74310777102836, 77.49790831353984	27.737333696765905, 77.48236983770744
	Mat	27.648509647246193, 77.71596017345011	27.64675845140139, 77.72703449042197	27.63740497714651, 77.72651599511782	27.631092608074695, 77.72900326216562	27.626378635293058, 77.72608316020502	27.624326892882124, 77.72024583697493	27.629346454803855, 77.71698541324365	27.624480400674525, 77.7132077558549	27.641818841301863, 77.70814673772938	27.64759826676443, 77.70711763079784

Table 7. List of the sampling sites for isolation of *Trichoderma* species along with corresponding GPS locations (latitude and longitude) in Aligarh and adjoining districts of Uttar Pradesh.

before the planting of rice seedlings. For seed treatment, paste of sorghum seeds was applied to the rice seeds (@10 g/kg seeds), 3 h before their sowing in the nursery bed.

Treatments and plant cultures

The seedlings of rice cultivar Swarna Sub-1 were transplanted in the two sets of pots (1 seedling/pot) filled with sterilized soil and FYM (4:1). In one set of pots *Trichoderma* isolates were applied as soil application (@10 g/kg soil), whereas, in other set rice seedlings raised from *Trichoderma* treated seeds were planted. Four watering treatments viz., 100, 75, 50 and 25% of recommended irrigation were maintained for each isolate of *Trichoderma* spp. with five replicate/pots. Under 100% watering, the pots were watered regularly on alternate days. For 75% watering pots were watered every 4th day, and for 50% and 25% watering every 6th and 8th day, respectively. Similar treatments for drought have also been maintained in other researches[50]. Further, the soil moisture percentage is an important matrix for determining the drought level, but it is very difficult to use it as a treatment because of it changes with the progress of time. Since, there were 11 *Trichoderma* isolates, a total of 480 Pots were maintained, which were arranged in a complete randomized block design on the roof top receiving uniform sunlight. At maturity, 3 months after transplanting, the plants were harvested to measure plant length (shoot and root) and fresh weight per plant. The treatments for each *Trichoderma* isolate for soil application or seed treatment were as follows.

$\mathbf{T_1}$ = Plant + 100% watering (control)
$\mathbf{T_2}$ = Plant + 75% watering
$\mathbf{T_3}$ = Plant + 50% watering
$\mathbf{T_4}$ = Plant + 25% watering
$\mathbf{T_5}$ = Plant + 100% watering + *Trichoderma* isolate
$\mathbf{T_6}$ = Plant + 75% watering + *Trichoderma* isolate
$\mathbf{T_7}$ = Plant + 50% watering + *Trichoderma* isolate
$\mathbf{T_8}$ = Plant + 25% watering + *Trichoderma* isolate

Statistical analysis

The data obtained from the five replicates were used to calculate the means. However, replicate data were used to analyze least significant difference (LSD at $P \leq 0.05$) with the help of Agricolae Package of R software[51]. Tukey HSD test was employed to mark significantly different treatments in the Tables. Two factor ANOVA was used to evaluate the data on plant growth variables.

Ethical statements

Both the authors have approved the content and authorship of the submitted manuscript, and all prevailing local, national, and international regulations and conventions, and scientific and ethical practices have been respected.

Plant compliance statement

The studies in this research article (plant field studies and experimental research, as well as the collection of plant material) were undertaken following laws and regulations pertinent to the institution.

Data availability

Both authors shall provide the datasets utilized and/or analyzed throughout the current work upon reasonable request.

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Author contributions

M.R.K. conceived the idea and critically checked the MS; M.H.S. executed the experiments, analyze the data and prepared the manuscript. Both authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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