

EFFECT OF NATIVE *TRICHODERMA* AS SEED TREATMENT ON GERMINATION AND SEEDLING PERFORMANCE OF LENTIL UNDER BIOTIC AND ABIOTIC STRESS CONDITIONS

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ABSTRACT

Physiological and water stress conditions substantially impede seed germination and the establishment of plant population. In 2020, a study was conducted in a completely randomized design (CRD) to identify potential *Trichoderma* strains that could alleviate physiological and water stress in germinating seeds. To create physiological stress, seeds were kept in an incubator at high relative humidity (90%) and temperature (40°C) for 20 days. Similarly, to induce water stress on germinating seeds, Polyethylene glycol (PEG) was applied in four concentrations (0, 5, 10, and 15%). Nine different isolates of *Trichoderma* were isolated from forest and agricultural soil and conidial suspension was applied to lentil seeds to deposit 10^7 cfu per gram of seeds. For each treatment, a total of one hundred seeds were placed on three layered moist blotter paper and incubated at $25 \pm 2^\circ\text{C}$. Germination was measured after every 24 hours. Under stress conditions, seeds treated with *Trichoderma* exhibited enhanced germination, root and shoot length, dry and fresh weight of root and shoot of seedlings as compared to untreated seeds. Highest seed germination under physiological stress and water stress was observed with *Trichoderma* isolates T₈₇, Forest soil and T₆₂, Darchula respectively. Isolate T₈₇, Forest soil demonstrated the highest vigor index of seedlings in stress conditions. Seed treated with *Trichoderma* isolates also displayed significant improvement in germination under biotic stress induced by *Sclerotium rolfsii*. *Trichoderma* isolate T₃₁, Banke showed highest germination under biotic stress. These findings highlight the potential of *Trichoderma* to enhance plant resilience and promote healthier growth under challenging environmental conditions.

Keywords: *Trichoderma*, Abiotic stress, Lentil, Seed germination, Seedling vigor

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INTRODUCTION

Lentil is an important winter season crop in Nepal, encompassing a substantial portion in terms of area (60%) and production (65%) among grain legumes (Darai et al., 2020). Despite the steady increase in lentil cultivation area and productivity over time, the national average yield still remains low at 1.2 tons per hectare. This is primarily attributed to a combination of factors such as lack of quality seeds, insufficient inputs, and technology, along with various biotic and abiotic constraints. Drought stress is one of the most widespread abiotic factors that curtail the growth and productivity of the crop in rainfed areas. Lentil cultivation often relies entirely on residual soil moisture within rice-based cropping systems rendering it highly sensitive to low soil moisture specially during seedling and flowering stages (Shrestha et al., 2006).

Seed quality determines the germination, plant population, and yield of the crop which is influenced by moisture content, relative humidity during seed storage, and storage duration. Throughout the storage period, a series of biochemical and physiological changes occur within seeds which results in a progressive decline in the vigor and viability of the seed (Marcos-Filho and McDonald, 1998; Chadordooz-Jeddi et al., 2015). As seeds undergo aging process, there is gradual reduction in germination rate, uniformity of seedling emergence, stress tolerance, and subsequent post-emergence seedling growth (Khan et al., 2003).

Trichoderma spp. are biocontrol agents effective in controlling plant pathogens and in addition to their biocontrol activity, they also promote growth and induces systemic resistance in the plant (Harman et al., 2004). They improve the decomposition of biomass and accelerate the uptake of nutrients thereby enhancing the overall crop productivity (Mehetre and Mukherjee, 2015). There are several reports of enhanced plant resistance to abiotic stress during plant growth attributed to improved root growth, increased water holding capacity, and increased potassium absorption (Bae et al., 2009; Yildirim et al., 2006). The present study was undertaken to assess the effectiveness of diverse *Trichoderma* isolates in improving germination and seedling performance under conditions of physiological stress (seed aging), water stress, and biotic stress (induced by *Sclerotium rolfsii*).

MATERIALS AND METHODS

Isolation of *Trichoderma* isolates

Trichoderma isolates were isolated from agricultural land and natural sal (*Shorea robusta*) forest by serial dilution method. Twenty microliter soil suspension was poured on *Trichoderma* selective medium (TSM). The plates were incubated at 25±2°C for seven days and individual colonies were isolated.

Isolation of *Sclerotium rolfsii*

S. rolfsii was isolated from infected lentil root. Potato Dextrose Agar (PDA) was used for isolation. Infected plant samples were placed in a moist chamber at 25°C for three days. Sclerotia formed in the plant samples were isolated and inoculated in PDA. The PDA plates were incubated at 25±2°C for four days for full growth of the pathogen.

Germination and seedling attributes under physiological stress conditions

Seeds of lentil variety 'Simrik' were used in the study. To induce physiological stress, the seeds were artificially aged (Kaewnaee et al., 2011). The seeds were sealed in plastic boxes and incubated at 40°C with 90% relative humidity for 20 days. The study encompassed nine different *Trichoderma* isolates viz. T₈₇, Forest soil; T₆₂, Darchula; T₇₀, Forest soil; T₇₇, Forest soil; T₆₁, Gorkha; T₅, Tanahun; T₄₉, Bhaktapur; T₃₁, Banke and T₈₄, Forest soil. A conidial suspension of *Trichoderma* was applied onto lentil seeds at the rate of 10 µl per gram to deposit 10⁷cfu per gram of seeds. Twenty-five seeds were placed per Petri dish with two layers of blotter paper moistened with distilled water. One hundred seeds formed one replication and four replications of each treatment were maintained. Control seeds were treated with an equal amount of distilled water. Observations were made at 24-hour intervals to check if the radicle had penetrated the seed coat, which marked the point of germination. Nine days later, the length, fresh weight, and dry weight of the radicle and hypocotyl of the seedlings were measured. The radicles and hypocotyls of seedlings were dried at 65°C for 48 hours and weighed.

Germination and seedling attributes under water stress conditions

To lower water potential, Polyethylene glycol (PEG) was added at the rate of 0, 5, 10, and 15% providing the osmotic potential of -0.001, -0.27, -0.54, and -0.81 MPa respectively. Seeds were treated with five *Trichoderma* isolates viz. T₈₇, Forest soil; T₆₂, Darchula; T₇₀, Forest soil; T₄₉, Bhaktapur and T₆₁, Gorkha. Seed treatment and incubation were similar as described above. PEG solution (2ml per plate) was added to the plate for nine days. Observations were made as described above.

Germination under biotic stress conditions

Lentil seeds were placed in a Petri dish containing *S. rolfsii* to assess the effect of *Trichoderma* on germination in the presence of the pathogen. The pathogen was cultured in the Petri dish for three days, then five seeds treated with *Trichoderma* isolates were placed in the Petri dish at equidistance. Four *Trichoderma* isolates viz., T₃₁, Banke, T₈₇, Forest soil, T₄₉, Bhaktapur, and T₁₈, Jumla were used for the study. Each treatment was replicated six times. Petri dishes were incubated in an incubator at 25 ± 1°C. Seed germination was recorded after every 24 hours and dead seedlings were counted. Plants were harvested 5 days after plating, and the length of hypocotyls was measured.

Statistical analysis

Both experiments were carried out in CRD with four replicates. Data were recorded after nine days and statistically analyzed using R-studio version 1.4.1717 and mean comparisons were made using DMRT.

RESULTS AND DISCUSSION

Effect of *Trichoderma* on lentil seed germination under biotic stress condition

The presence of *S. rolfisii* significantly reduced the germination and epicotyl length in seeds even when treated with *Trichoderma* isolates (Table 1). In contrast, untreated lentil seeds did not germinate at all. Seeds treated with *Trichoderma* isolates T₃₁, Banke exhibited the highest germination percentage (86.67%). Similarly, *Trichoderma* isolate T₈₇, Forest soil demonstrated the longest epicotyl length (2.05 cm) as compared to other isolates. Despite the beneficial impact of *Trichoderma* seed treatment on seed germination, the seedlings' mortality due to *S. rolfisii* infection occurred.

Table 1. Effect of *Trichoderma* isolates on germination and epicotyl length of lentil seeds under *S. rolfisii* infection five days after incubation

<i>Trichoderma</i> isolates	Germination (%)	Epicotyl length (cm)
T ₃₁ , Banke	86.67 ^a	1.60 ^a
T ₄₀ , Jumla	76.67 ^a	1.76 ^a
T ₈₇ , Forest soil	70.00 ^a	2.05 ^a
T ₄₉ , Bhaktapur	43.33 ^b	1.31 ^a
Control	0.00 ^c	0.00 ^b
S.Em±	0.35	0.13
F- test	***	***
LSD (=0.05)	21.51	0.39

Figures followed by the same letter in column are not significantly different by DMRT.

Effect of *Trichoderma* under physiological stress conditions

Nine native *Trichoderma* isolates were tested *in vitro* to study their effect on lentil seed germination and the characteristics of emerging seedlings. The results revealed that the seed aging process of seeds had a negative impact on their germination. Application of *Trichoderma* isolate T₈₇, Forest soil mitigated the seed aging leading to the highest seed germination (84%) and root length (5.55cm) as compared to untreated aged seed (Table 2). Among the treatments, the longest shoot length was observed in seed treated with *Trichoderma* isolate T₆₂, Darchula (8.07cm). However, these results were not significantly different from the control treatment. Seedlings

arising from aged seeds that were treated with *Trichoderma* exhibited superior growth in terms of hypocotyl and radicle length, greater accumulation of biomass in terms of shoot and root fresh and dry weight, and enhanced seedling vigor as compared to untreated seedlings. *Trichoderma* isolate T₆₂, Darchula significantly improved the fresh root weight of seedlings (267.5mg) but it was statistically at par with other *Trichoderma* isolates except T₇₀, Forest soil (157.5mg) and T₅, Tanahun (167.5mg). This isolate also displayed the highest dry root (42 mg) and shoot weight (68 mg) as compared to the control. Among the nine *Trichoderma* isolates, T₃₁, Banke isolate exhibited a significant enhancement of fresh shoot weight (440 mg). Remarkably, the highest vigor index was achieved with aged lentil seeds treated with *Trichoderma* isolate T₈₇, Forest soil (8.86).

Table 2. Effect of *Trichoderma* seed treatment on the growth of lentil seedlings under physiological stress condition

<i>Trichoderma</i> isolates	G (%)	RL (cm)	SL (cm)	FRW (mg)	FSW (mg)	DRW (mg)	DSW (mg)	VI
T ₈₇ , Forest soil	84	5.55	7.38	250.0 ^{ab}	420.0 ^{abc}	39.00 ^{ab}	66.75 ^{ab}	8.86 ^a
T ₆₂ , Darchula	77	5.13	8.07	267.5 ^a	437.5 ^{ab}	42.00 ^a	68.50 ^a	8.58 ^{ab}
T ₇₀ , Forest soil	74	3.13	5.83	157.5 ^{cd}	325.0 ^{cd}	25.00 ^{cd}	51.00 ^{bc}	5.60 ^{cd}
T ₇₇ , Forest soil	73	4.88	6.14	200.0 ^{a-c}	342.5 ^{a-d}	31.00 ^{a-c}	53.25 ^{a-c}	6.15 ^{b-d}
T ₆₁ , Gorkha	79	4.40	6.82	212.5 ^{a-c}	395.0 ^{a-c}	33.50 ^{a-c}	62.00 ^{ab}	7.48 ^{a-c}
T ₅ , Tanahun	73	5.09	6.51	167.5 ^{b-d}	337.5 ^{b-d}	26.25 ^{cd}	52.75 ^{a-c}	5.80 ^{cd}
T ₄₉ , Bhaktapur	74	3.74	6.71	187.5 ^{a-d}	355.0 ^{a-d}	29.25 ^{b-d}	55.75 ^{a-c}	6.24 ^{b-d}
T ₃₁ , Banke	65	4.74	6.98	205.0 ^{a-c}	442.5 ^a	31.75 ^{a-c}	69.25 ^a	6.31 ^{b-d}
T ₈₄ , Forest soil	71	4.10	6.99	212.5 ^{a-c}	422.5 ^{a-c}	33.00 ^{a-c}	66.00 ^{ab}	6.97 ^{a-c}
Control	67	2.95	6.06	110.0 ^d	280.0 ^d	18.25 ^d	44.00 ^c	4.17 ^d
S.Em±	5.02	0.67	0.55	24.3	31.28	3.83	4.97	0.74
F- test	ns	ns	ns	*	*	**	*	**
LSD (=0.05)				70.52	90.78	11.13	14.43	2.17

Figures followed by the same letter in the column are not significantly different by DMRT. G:Germination, RL:Root length, SL:Shoot length, FRW:Fresh root weight, FSW:Fresh shoot weight, DRW:Dry root weight, DSW:Dry shoot weight, VI:Vigor index

Effect of *Trichoderma* under water stress conditions

Effect on germination of lentil seeds

Trichoderma isolates had a significant effect on seed germination under water stress condition (Fig. 1 and 2). It was evident that the germination percentage of seeds decreased as the concentration of PEG increased as a result of reduced osmotic potential. This pattern persisted regardless of whether the seeds were treated with *Trichoderma* or not. Additionally, the response of seed germination varied with

different *Trichoderma* isolates under varying levels of water stress. Notably, the highest seed germination was recorded when seeds were exposed to a PEG concentration of 0%, and seed treated with *Trichoderma* isolate T₆₂, Darchula (Fig. 1 and 2 respectively).

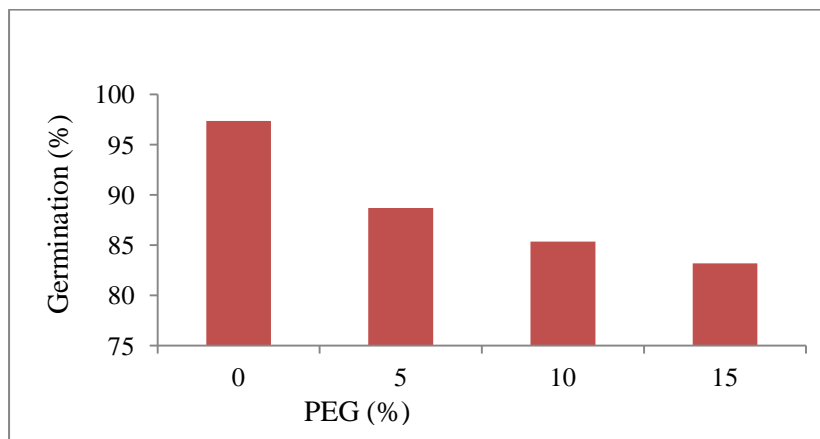


Figure 1. Effect of PEG concentration on germination percentage of lentil seeds.

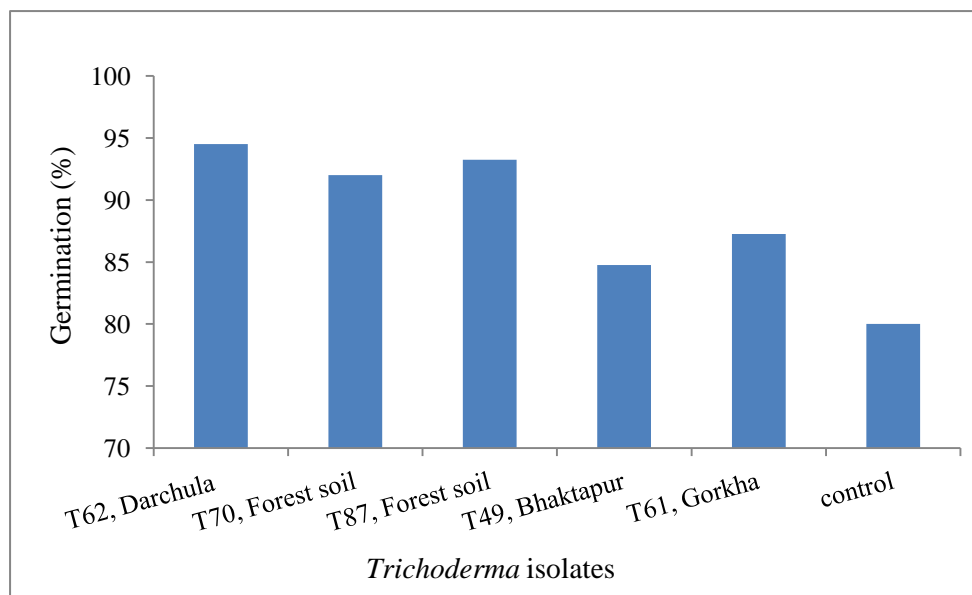


Figure 2. Effect of *Trichoderma* seed treatment on germination of lentil seed under water stress condition.

Effect on root and shoot length of lentil seedlings

Seed treatment with *Trichoderma* showed a positive effect on the root and shoot length of seedlings. As water potential decreased, an increase in root length was observed (Table 3). The longest root length was measured 9.64 cm in case control treatment exposed to 15% PEG. For *Trichoderma* isolates T₆₁, Gorkha (8.81cm), T₄₉, Bhaktapur (9.44cm), and T₆₂, Darchula (8.05cm), the longest root lengths were recorded at 10% PEG. However, for *Trichoderma* isolate T₇₀, Forest soil, the longest root length (5.70cm) was observed in 10% PEG, and was statistically at par with 5% and 15% PEG. Conversely, isolate T₈₇, Forest soil (6.43cm) showed the longest root length at 15% PEG, but this measurement was similar to the results at 5% and 10% PEG.

An increase in percentage of PEG led to a decrease in the shoot length (Table 3). The longest shoot length (7.45 cm) was found in *Trichoderma* isolate T₈₇, Forest soil which was statistically similar to other *Trichoderma* isolates and the control for 0% PEG. However, at 15% PEG, seedlings treated with *Trichoderma* isolates exhibited shorter shoot length as compared to untreated seedlings. These findings indicate that seedlings arising from *Trichoderma*-treated seeds exhibit reduced shoot lengths compared to untreated seeds under reduced water potential.

Table 3. Effect of *Trichoderma* seed treatment on root and shoot length of seedling under water stress condition.

<i>Trichoderma</i> isolates	PEG%							
	Root length (cm)				Shoot length (cm)			
	0	5	10	15	0	5	10	15
T ₈₇ , Forest soil	4.21 ^{g-i}	5.10 ^{d-i}	5.71 ^{d-h}	6.43 ^{c-f}	7.45 ^a	3.91 ^{h-j}	4.05 ^{g-j}	3.84 ^{h-j}
T ₆₂ , Darchula	3.75 ⁱ	4.66 ^{f-i}	8.05 ^{a-c}	6.11 ^{d-g}	6.90 ^{ab}	3.80 ^{h-j}	5.44 ^{c-g}	3.90 ^{h-j}
T ₇₀ , Forest soil	3.74 ⁱ	5.45 ^{d-i}	5.70 ^{d-h}	5.42 ^{d-i}	6.21 ^{a-d}	4.09 ^{g-j}	3.71 ^{ij}	3.44 ^j
T ₄₉ , Bhaktapur	4.92 ^{e-i}	4.94 ^{e-i}	9.44 ^a	6.10 ^{b-d}	6.67 ^{a-c}	5.22 ^{d-h}	7.18 ^{ab}	5.09 ^{d-i}
T ₆₁ , Gorkha	3.89 ^{hi}	5.09 ^{d-i}	8.81 ^a	6.76 ^{b-e}	6.04 ^{a-e}	4.67 ^{e-j}	6.70 ^{a-c}	4.47 ^{g-j}
Control	3.59 ⁱ	6.00 ^{d-g}	8.47 ^{ab}	9.64 ^a	6.37 ^{a-d}	4.54 ^{f-j}	6.08 ^{a-e}	5.91 ^{b-f}
S.Em±	0.57				0.43			
F- test	***				***			
LSD (=0.05)	1.63				1.22			

Figures followed by the same letter in the column are not significantly different by DMRT.

Effects on fresh root and shoot weights of seedlings

As the concentration of PEG increased, there was a gradual reduction in the fresh weights of both the root and shoot in lentil seedlings, as outlined in Table 4. In the case

of 0% PEG, seed treatment with *Trichoderma* exhibited a significant effect on the fresh root and shoot weight. The highest fresh root weight was recorded in case of *Trichoderma* isolate T₈₇, Forest soil (410.0mg) which was significantly at par with T₄₉, Bhaktapur (430.0mg), T₇₀, Forest soil (397.25mg), and T₆₂, Darchula (370.0mg). However, the lowest fresh root was recorded in the control treatment (92.75 mg) at 15% PEG.

Similarly, the highest fresh shoot weight was recorded in seedlings produced from isolate T₇₀, Forest soil (461.25mg), exhibiting statistical similarity to T₄₉, Bhaktapur (452.0mg), T₆₂, Darchula (426.0mg), and T₈₇, Forest soil (412.75mg) under 0% PEG condition. In the context of 5% PEG, isolates T₄₉, Bhaktapur (330.0mg) and T₆₁, Gorkha (355.0mg) displayed significantly higher root and shoot weight than other isolates. While, *Trichoderma* seed treatment significantly increased root and shoot weight under 0% and 5% PEG, these isolates did not manifest a statistically significant effect under higher PEG concentrations.

Table 4. Effect of *Trichoderma* seed treatment on fresh root and shoot weight of seedling under water stress condition.

<i>Trichoderma</i> isolates	PEG%							
	Fresh root weight(mg)				Fresh shoot weight(mg)			
	0	5	10	15	0	5	10	15
T ₈₇ , Forest soil	410.0 ^a	143.5 ^{d-f}	112.5 ^{ef}	100.8 ^{ef}	412.8 ^{a-c}	241.8 ^{fg}	225.0 ^g	202.5 ^g
T ₆₂ , Darchula	370.0 ^{a-c}	139.0 ^{ef}	112.5 ^{ef}	130.0 ^{ef}	426.0 ^{ab}	239.0 ^{fg}	210.0 ^g	200.0 ^g
T ₇₀ , Forest soil	397.3 ^{ab}	124.8 ^{ef}	135.0 ^{ef}	197.5 ^{ef}	461.3 ^a	237.3 ^{fg}	217.5 ^g	186.5 ^g
T ₄₉ , Bhaktapur	430.0 ^a	162.5 ^{d-f}	103.3 ^{ef}	112.5 ^{ef}	452.0 ^{ab}	330.0 ^{de}	302.5 ^{ef}	300.3 ^{ef}
T ₆₁ , Gorkha	319.8 ^c	220.0 ^d	160.0 ^{d-f}	127.5 ^{ef}	347.8 ^{c-e}	355.0 ^{c-e}	242.5 ^{fg}	200.0 ^g
Control	326.75 ^{bc}	182.00 ^{de}	122.50 ^{ef}	92.750 ^f	392.75 ^{b-d}	236.75 ^{fg}	202.50 ^g	186.0 ^g
S.Em±	24.93				21.64			
F- test	***				***			
LSD (=0.05)	70.36				61.06			

Figures followed by the same letter in column are not significantly different by DMRT.

Effects on dry roots and shoots weights of seedlings

Water stress had a pronounced effect on reducing the dry weights of both root and shoot (Table 5). However, seed treatment with *Trichoderma* enhanced dry root and shoot weights, except T₆₁, Gorkha under 0% PEG condition. As the concentration of PEG increased, both dry root and shoot weights declined. However, at PEG concentrations of 10% and 15%, the application of, *Trichoderma* to seeds did not exhibit a discernible effect.

In general, the vigor index decreased with an increase in PEG concentration (Table 5). At 0% PEG concentration, the lowest vigor index was recorded in seeds treated with *Trichoderma* isolate T₆₁, Gorkha isolate (10.02mg) and in the control (10.38mg). At 5% PEG concentration, *Trichoderma* isolates T₄₉, Bhaktapur (6.71

mg) and T₆₁, Gorkha (7.92 mg) demonstrated a superior vigor index compared to other isolates. Notably, in the control, the vigor index was lowest at 15% PEG though the result was statistically similar to 10% PEG.

Table 5. Effect of *Trichoderma* seed treatment on dry root and shoot weight of seedling under water stress condition.

<i>Trichoderma</i> isolates	PEG%											
	Dry root weight (mg)				Dry shoot weight (mg)				Vigor index			
	0	5	10	15	0	5	10	15	0	5	10	15
T ₈₇ , Forest soil	65.05 ^a	22.43 ^{d-f}	17.57 ^{ef}	15.77 ^{ef}	64.78 ^{a-c}	37.84 ^{fg}	35.21 ^g	31.72 ^g	12.98 ^a	5.66 ^{de}	4.91 ^{d-f}	4.08 ^{ef}
T ₆₂ , Darchula	57.30 ^{a-}	21.78 ^{ef}	17.52 ^{ef}	20.38 ^{ef}	66.69 ^{ab}	37.43 ^{fg}	32.82 ^g	31.29 ^g	12.28 ^a	5.68 ^{de}	4.59 ^{d-f}	4.75 ^{d-f}
T ₇₀ , Forest soil	62.03 ^{ab}	19.63 ^{ef}	16.27 ^{ef}	15.31 ^{ef}	72.01 ^a	37.19 ^{fg}	34.19 ^g	29.24 ^g	13.27 ^a	5.09 ^{d-f}	4.82 ^{d-f}	3.80 ^{ef}
T ₄₉ , Bhaktapur	67.25 ^a	25.52 ^{def}	21.07 ^{ef}	17.66 ^{ef}	70.64 ^{ab}	51.76 ^{de}	47.36 ^{ef}	47.06 ^{ef}	13.55 ^a	6.71 ^{cd}	5.31 ^{d-f}	5.01 ^{d-f}
T ₆₁ , Gorkha	49.97 ^c	34.52 ^d	25.02 ^{def}	19.94 ^{ef}	54.27 ^{de}	55.65 ^{c-e}	38.04 ^{fg}	31.39 ^g	10.02 ^b	7.92 ^c	5.18 ^{d-f}	4.27 ^{ef}
Control	51.09 ^{bc}	28.26 ^{de}	19.10 ^{ef}	14.55 ^f	61.38 ^{b-d}	37.05 ^{fg}	31.66 ^g	29.36 ^g	10.38 ^b	5.22 ^{d-f}	3.72 ^{ef}	3.37 ^f
S.Em±	3.87				3.35				0.63			
F- test	*				***				*			
LSD (=0.05)	10.92				9.46				1.78			

Figures followed by the same letter in column are not significantly different by DMRT.

Plants typically experience a number of abiotic stresses that have a detrimental impact on their development, growth, and yield. These stresses lead to decreased seed germination, reduced seedling vigor, and hindered plant emergence, ultimately causing a reduction in seed yield (Singh et al., 2016). Several *Trichoderma* species possess the ability to alleviate abiotic stresses, enhanced plant vigor and growth (Hermosa et al., 2012; Shoresh et al., 2010). The stresses examined here, resulted in lower fresh and dry root and shoot weights of lentil seedlings. Conversely, *Trichoderma*-treated seed displayed superior fresh and dry root and shoot weights of seedlings.

Evidence from previous studies supports the notion of *Trichoderma*'s potential to enhance plant resilience. Bae et al. (2009) and Yusnawan et al. (2019) reported that *Trichoderma* sp. increased tolerance of cocoa and soybean plants to water stress by promoting root growth. Similar results have been reported in diverse crop seedlings treated with *Trichoderma* spp. (Alfano et al., 2006; Mastauri et al., 2010; Nayaka et al., 2008; Swain et al., 2021; Tančić-Živanov et al., 2020). Similarly, Halifu et al. (2019) reported that the inoculation of *Pinus* with *T. harzianum* and *T. virens* enhanced root structure index and seedling biomass. Mastauri et al. (2010) demonstrated the ability of *Trichoderma* strains to confer plant tolerance against physiological stress. Mastouri et al. (2012) demonstrated that *T. harzianum*

contributes to physiological protection against oxidative damage in plants, increasing seedling vigor and mitigating stress.

In this study, seedlings produced from treated seeds exhibited superior seedling vigor in both physiological and water stress conditions compared to untreated seedlings. Swain et al. (2018) reported an elevated vigor index of rice treated with *Trichoderma*. Furthermore, according to Cai et al. (2013), *Trichoderma* spp. produce different phenolic compounds and harzianolide, the secondary metabolite, which contributes to seedlings' vigor. Along with the previously mentioned mechanism, *Trichoderma* has the ability to improve plant growth under challenging abiotic conditions by reducing harmful elevated levels of ethylene (Brotman et al., 2013). Moreover, apart from abiotic stress tolerance, *Trichoderma* spp. can instigate systemic resistance in plants resulting in tolerance against other fungal pathogens. In this study, untreated seeds failed to germinate in the presence of *S. rolfsii* in control, whereas germination was observed when seeds were treated with *Trichoderma* isolates. In recent studies, it has been observed that *Trichoderma* spp. can reprogram plant gene expression, thereby priming plants for pathogen defense and resulting in induced systemic resistance (Shoresh et al., 2010).

CONCLUSION

The application of *Trichoderma* spp. enhances stress tolerance in lentil. Application of *Trichoderma* spp. into the field would enhance the crop yield and have the potential to mitigate the different challenging biotic and abiotic stresses. Seed treatment with *Trichoderma* exhibited a positive impact on seed germination and seedling characteristics under physiological and water stress conditions. These findings underscore the potential of *Trichoderma* as a stress-alleviating agent, promoting seed germination, seedling growth and overall plant vigor under diverse stress conditions. With the reference to research findings, *Trichoderma* isolate T₈₇, Forest soil is a promising isolate and can be used to ameliorate the various stresses in field conditions.

REFERENCES

- Alfano, G., Ivey, M.L., Cakir, C., Bos, J.I.B., Miller, S.A., Madden, L.V., Kamoun, S. and Hoitink, H.A.J. (2007). Systemic modulation of gene expression in tomato by *Trichoderma hamatum* 382. *Phytopathology*, 97(4): 429-437.
- Bae, H., Sicher, R.C., Kim, M.S., Kim, S.H., Strem, M.D., Melnick, R.L. and Bailey, B. A. (2009). The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. *Journal of experimental botany*, 60(11): 3279-3295.
- Brotman, Y., Landau, U., Cuadros-Inostroza, A., Takayuki, T., Fernie, A.R., Chet, I., Viterbo, A. and Willmitzer, L. (2013). *Trichoderma*-plant root colonization: escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance. *PLoS pathogens*, 9(3): p.e1003221.

- Cai, F., Yu, G., Wang, P., Wei, Z., Fu, L., Shen, Q. and Chen, W. (2013). Harzianolide, a novel plant growth regulator and systemic resistance elicitor from *Trichoderma harzianum*. *Plant Physiology and Biochemistry*, 73: 106-113.
- Chadordooz-Jeddi, A., Ghassemi-Golezani, K. and Zehtab-Salmasi, S. (2015). The impact of seed size and aging on physiological performance of lentil under water stress. *Journal of Plant Physiology and Breeding*, 5(1): 13-21.
- Gairhe, S., Timsina, K.P., Adhikari, S.P., Sapkota, B. and Sapkota, S. (2023). Lentil Seed System in Nepal. In *Proceeding of 32nd National Winter Crop Workshop in Nepal* (pp. 533-540).
- Halifu, S., Deng, X., Song, X. and Song, R. (2019). Effects of two *Trichoderma* strains on plant growth, rhizosphere soil nutrients, and fungal community of *Pinus sylvestris* var. *mongolica* annual seedlings. *Forests*, 10(9): 758.
- Harman, G. E., Howell, C. R., Viterbo, A., Chet, I. and Lorito, M. (2004). *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nature reviews microbiology*, 2(1): 43-56.
- Hermosa, R., Viterbo, A., Chet, I. and Monte, E. (2012). Plant-beneficial effects of *Trichoderma* and of its genes. *Microbiology*, 158(1): 17-25.
- Kaewnaree, P., Vichitphan, S., Klanrit, P., Siri, B. and Vichitphan, K. (2011). Effect of accelerated aging process on seed quality and biochemical changes in sweet pepper (*Capsicum annum* Linn.) seeds. *Biotechnology*, 10(2): 175-182.
- Khan, M. M., Iqbal, M. J., Abbas, M. and Usman, M. (2003). Effect of aging on viability, vigour and chromosomal damage in pea (*Pisum sativum* L.) seeds. *Pakistan Journal of Agricultural Science*, 40: 50-54.
- Marcos-filho, J. and McDonald, M. B. (1998). Sensitivity of RAPD analysis, germination and vigor tests to detect the intensity of deterioration of naturally and artificially aged soybean seeds. *Seed Science and Technology*, 26(1): 141-157.
- Mastouri, F., Björkman, T. and Harman, G. E. (2012). *Trichoderma harzianum* enhances antioxidant defense of tomato seedlings and resistance to water deficit. *Molecular plant-microbe interactions*, 25(9): 1264-1271.
- Mehetre, S.T. and Mukherjee, P.K. (2015). *Trichoderma* improves nutrient use efficiency in crop plants. *Nutrient use efficiency: from basics to advances*, 173-180.
- Chandra Nayaka, S., Niranjana, S.R., Uday Shankar, A.C., Niranjana Raj, S., Reddy, M.S., Prakash, H.S. and Mortensen, C.N. (2010). Seed biopriming with novel strain of *Trichoderma harzianum* for the control of toxigenic *Fusarium verticillioides* and fumonisins in maize. *Archives of Phytopathology and Plant Protection*, 43(3): 264-282.
- Shoresh, M., Harman, G.E. and Mastouri, F. (2010). Induced systemic resistance and plant responses to fungal biocontrol agents. *Annual Review of Phytopathol*, 48: 21-43. <https://doi.org/10.1146/annurev-phyto-073009-114450>
- Shrestha, R., Turner, N.C., Siddiqu, K.H.M. and Turner, D.W. (2006). Physiological and seed yield responses to water deficit among lentil genotypes from diverse origins. *Australian Journal of Agricultural Research*, 57: 903-915.

- Singh, D.P., Singh, H.B. and Prabha, R. (2016). Microbial inoculants in sustainable agricultural productivity. Springer (India).
- Swain, H., Adak, T., Mukherjee, A.K., Mukherjee, P.K., Bhattacharyya, P., Behera, S., Bagchi, T.B., Patro, R., Khandual, A., Bag, M.K. and Dangar, T.K. (2018). Novel *Trichoderma* strains isolated from tree barks as potential biocontrol agents and biofertilizers for direct seeded rice. *Microbiological research*, 214: 83-90.
- Swain, H., Adak, T., Mukherjee, A.K., Sarangi, S., Samal, P., Khandual, A., Jena, R., Bhattacharyya, P., Naik, S.K., Mehetre, S.T. and Baite, M.S. (2021). Seed biopriming with *Trichoderma* strains isolated from tree bark improves plant growth, antioxidative defense system in rice and enhance straw degradation capacity. *Frontiers in Microbiology*, 12: 633881. <https://doi.org/10.3389/fmicb.2021.633881>
- Tančić-Živanov, S., Medić-Pap, S., Danojević, D. and Prvulović, D. (2020). Effect of *Trichoderma* spp. on growth promotion and antioxidative activity of pepper seedlings. *Brazilian Archives of Biology and Technology*, 63: e20180659. <https://doi.org/10.1590/1678-4324-2020180659>
- Yildirim, E., Taylor, A.G. and Spittler, T.D. (2006). Ameliorative effects of biological treatments on growth of squash plants under salt stress. *Scientia Horticulturae*, 111: 1-6.
- Yusnawan, E., Inayati, A. and Baliadi, Y. (2019). Effect of soybean seed treatment with *Trichoderma virens* on its growth and total phenolic content. AIP Conference Proceedings. 03 July 2019.