RESEARCH ARTICLE

The impact of *Trichoderma* isolates on seed germination, vigor index, and health of chilli cultivars

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Chilli (*Capsicum annuum* L.) is a highly consumed and economically significant crop. The *Trichoderma* genus, renowned for its role as a biological control agent, exhibits diverse mechanisms influencing plant growth and defense. This research investigates the effect of *Trichoderma* isolates on the germination percentage, vigor index, and growth parameters of various chilli varieties. The study isolates indigenous *Trichoderma* spp. from rhizosphere soils and assesses their culture filtrates' effects on chilli seeds. Significant enhancements were observed in seed germination percentage, radical and plumule length, as well as seedling fresh and dry weight under *Trichoderma* treatments, particularly TRNU-1 and TRNU-2. Among the all the verities of chilli Kashmiri Long found the highest seed germination (64.67%), seedling length (8.88 cm), 10 seedling fresh weight (0.44 g) and vigour index-I (608.27), Pusha Jwala found the highest 10 seedling dry weight (0.35 g) and vigour index-II (16.34) and UHFC-12 found the highest radical length (4.47 cm) and plumule (4.70 cm). These findings highlight the potential of *Trichoderma* as a biocontrol agent and growth promoter in chilli cultivation, emphasizing its potential for sustainable agriculture and improved crop productivity.

Key Words: *Chilli*; Trichoderma; *Biological control agent*; *Varieties*; *Culture filtrates*

INTRODUCTION

Asia shows the maximum chilli (*Capsicum annuum* L.) production worldwide, of which India is the largest producer and consumer of this spice [1]. Chilli, renowned as the "red pepper," holds significant prominence as a vital vegetable and spice crop cultivated worldwide [2]. Indian chilli peppers are renowned for their distinctive spiciness and vibrant color in the commercial world. Several fungal strains recognized for their function as biological control agents belong to the *Trichoderma* genus [3]. *Trichoderma* isolates often associate with root ecosystems and plant roots. *Trichoderma* isolates have been categorized by some scientists as opportunistic a virulent plant symbiont that can colonize plant roots *via* processes similar to those of mycorrhizal fungi. Moreover, they generate compounds that stimulate plant growth and defense mechanisms [4]. The application of *Trichoderma* spp. not only demonstrates antagonistic effects on plant pathogens but also fosters positive impacts on plant growth and yield in various vegetable crops [5-8]. In agricultural fields, the introduction of *Trichoderma hamatum* or *Trichoderma koningii* can result in an increase in crop productivity by up to 30 percent.

Recent findings have revealed certain *Trichoderma* strains that can produce cytokinin like molecules such as zeatin and gibberellin GA3 or GA3-related compounds. Regulated production of these substances has the potential to improve bio-fertilization [9]. Furthermore, *Trichoderma* isolates change their immediate environment by secreting organic acids such as fumaric, citric or gluconic acid, which are produced by the metabolism of carbon sources, mainly glucose. Acids help phosphates, micronutrients, and mineral cations including iron, manganese, and magnesium become soluble [4]. These advantageous fungi therefore display a variety of mechanisms of action. The purpose of this study is to assess, under lab settings, how culture filtrates from various *Trichoderma* strains affect the percentage of chili seeds that germinate.

MATERIALS AND METHODS

The study titled "The impact of *Trichoderma* isolates on seed germination, vigor index, and health of chilli cultivars" was conducted in the Plant Pathology laboratory in collaboration with the Plant Breeding department at RNB Global University, Bikaner, in 2023. *Trichoderma* isolates were isolated and preserved for the study.

Isolation of native *Trichoderma* spp. isolates

To isolate indigenous *Trichoderma* spp. from the rhizosphere soils, samples were collected from chilli fields. Each soil sample, weighing approximately 250-300 g, was carefully gathered in a polythene bag and appropriately labeled with details including the host, location and soil type. Specifically, the soil was procured from the immediate area surrounding the root zone (rhizosphere soil) and then transported to the laboratory for subsequent analysis. Using the conventional technique of serial dilution plating [10], the samples were processed, utilizing PDA medium for the purpose of isolation. The fungal colonies that appeared on the plates were subsequently sub-cultured and purified on PDA slants to enable further investigations [11]. The count of the developed colonies was carefully recorded, and the *Trichoderma* population was determined in relation to the other colonies present.

Preparation of culture filtrates (*Trichoderma*)

Richard's solution, 200 ml of which contained 1.0 g of KNO_3, 0.5 g of KH_2PO_4, 0.25 g of $MgSO_47H_2O$, 34 g of glucose, and trace amounts of $FeCl_3$ in 1 liter of distilled water that had been adjusted to pH 6.5, was made and then transferred into 500 ml conical flasks. The flasks were then autoclaved for 15 min at 121°C and 1.05 kg/cm^2 of pressure. Six agar discs (6 mm) per strain and four replications were added to a flask holding the media for each strain of *Trichoderma*. The flasks were then kept at 28°C and rotated at 100 rpm in a Gallenkamp orbital incubator, following the instructions provided by Dennis and Webster [12]. The culture filtrates were collected after 30 days of incubation, concentrated using a vacuum evaporator at 38-40°C to around 50%, and then filtered through a membrane filter that had been sterilized.

Seed selection and treatment

The seed of chilli variety PusaJwala, Kashmiri Long, Pusa Sadabahar and UHFC12-4 was used and collected from local market of Bikaner (Rajasthan). The seeds, which were one-year-old and stored at 5°C, exhibited a standard germination rate of 98%. Seeds without any visible cracks or deformations were carefully selected and surface sterilized for 10 min with 1% sodium hypochlorite solution. Subsequently, the seeds were thoroughly rinsed three times with sterilized distilled water and left to air dry. A seed coating solution was prepared using *Trichoderma* culture filtrates, to which 2% starch (w/v) was added as an additive. The dry chilli seeds were immersed in the culture filtrates supplemented with 2% starch (w/v) for each *Trichoderma* isolate, with an immersion period of 1-2 min. For the untreated control seeds, they were dipped in a 2% starch solution, and for the water control,

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seeds were immersed in water. Afterward, the seeds were allowed to air dry in a laminar airflow hood and then placed in Petri plates lined with two layers of Whatsman filter paper soaked in sterile distilled water. In each Petri plate, 25 seeds were arranged and placed in germinator and there were four replications. The germination of seeds was subsequently compared with the control treatments (seeds treated with 2% starch and seeds treated with water). The percentage of seed germination and the vigor index were recorded after a period ranging from 3 to 8 days.

Standard germination (%)

In each Petri plate, 25 seeds were arranged and placed in the germinator at 25°C ± 1°C for 14 days [13]. The seedlings were assessed at regular intervals, and after fourteen days, the per cent of normal seedlings were counted as germination.

$$Germination\% = (Normal\ seedlings)/(Total\ no\ of\ seeds) \times 100$$

Radical length (cm): During the final count in each replication, the radical length of 10 randomly selected seedlings was determined using the measurement scale.

Plumule length (cm): On the last count in each replication, the plumule length of 10 randomly chosen seedlings was measured using a measuring scale.

Seedling length (cm): Ten randomly chosen seedlings were counted at the end of each replication, and the length of each seedling was measured using a measuring scale.

Fresh weight of seedlings (g): The fresh weight of the seedlings was evaluated following the final count in the standard germination test, conducted over a period of 14 days. Ten healthy seedlings were selected at random from each replication of the germination test.

Dry weight of seedlings (g): Following the initial weighing, the seedlings were subjected to a 48 h drying period in an oven set at a temperature range of 65-70°C. The dried seedlings were subsequently weighed to determine the average seedling dry weight.

Seedling vigor index

The seedling vigor index was calculated using two distinct methods, as described by Abdul-Baki et al., [14].

Seedling vigor index I: The seedling vigor index I was derived using the formula:

$$Seedling\ vigor\ index\ I = Standard\ germination\ (\%) \times Seedling\ length\ (cm)$$

Seedling vigor index II: The seedling vigor index II was calculated using the formula:

$$Seedling\ vigor\ index\ II = Standard\ germination\ (\%) \times Seedling\ dry\ weight\ (g)$$

RESULTS AND DISCUSSION

The impact of culture filtrates from three *Trichoderma* isolates on the laboratory germination of Chilli seeds is presented in tabular form. Statistical analysis of the data revealed significant variations among the treatments, with a significance level of $P \leq 0.01$. The mean standard germination ranged from 67.17% to 54.5% across the genotypes with significant difference (Table 1). The maximum standard germination among mean value of chilli varieties was observed in the case of Kashmiri Long (64.67%) followed by Pusa Jwala (63.06%), Pusa Sadabahar (62.21%) whereas the minimum standard germination was recorded in (UHFC-12-4%). Among all the treatments highest mean value of germination % was found in TRNU-1 (65.02) and lowest was recorded in Control (57.17). Results showed that interaction due to variety and treatments had significant difference for standard germination (%). Germination percentage happens to be one of the most important characteristics of seed to be commercially used. Germination seems to be a biological process depending on several factors including the differential behavior of genotypes. Standard germination is related to the level of viability of seeds lot in a particular genotype. Differential response of the seed lot of the genotypes to standard germination might be due variable genetic makeup of the genotypes. The findings of present investigation on standard germination could be compared with reports of earlier works viz., Natesh et al., [15], Hunje et al., [16], Christinal et al., [17] and Kivadasannavar et al., [18] in chilli. Similarly, the early germination and highest germination percentages demonstrated by five *Trichoderma* strains have been documented by various researchers in different plant species Hanson [19], Mishra et al., [20] and Oyarbide et al., [21]. Additionally, several other species such as T. longipile and T. tomentosum have been proven to stimulate plant growth [22].

TABLE 1

Effect of isolates (*Trichoderma*) on seed germination of chilli varieties

Treatment	Varieties				
	Pusa jwala	Kashmiri long	Pusa sadabahar	UHFC 12-4	Mean
TRNU-1	4.25	5.1	4.313	4.953	4.654
TRNU-2	4.823	4.37	4.303	4.778	4.568
TRNU-3	4.125	3.88	4.168	4.635	4.202
Control	3.953	3.408	3.453	3.55	3.591
Mean	4.29	4.189	4.06	4.479	
	Variety	Treatment	Variety × Treatment		
SE ±	0.071	0.071	0.142		
CD (1%)	0.143	0.143	0.287		

The radical length is associated with ability of seedlings to establish in moisture and nutrient rich zone of soil. Therefore, this trait may be linked with stress tolerance ability of genotypes. The mean radical length ranged from 4.47 to 4.06 across the genotypes with significant difference (Table 2). The maximum radical length among mean value of chilli varieties was observed in the case of UHFC 12-4 (4.47 cm) followed by Pusa Jwala (4.29 cm), Kashmiri Long (4.06 cm) whereas the minimum radical lenght was recorded in Pusa Sadabahar (4.06 cm). Among all the treatments highest mean value of radical length was found in TRNU-1 (4.65 cm) and lowest was recorded in control (3.59 cm). The study indicated that the interaction due to variety and treatments had significant difference for radical length. Longer root length in plants indicates greater tolerance of the genotypes to moisture stress. Different genotypes of chilli showed high variability for seedling radical length. Corresponding results on variability in radical length have also been reported by Dhanelappagol et al., [23], Hunje et al., [16], Manjunath et al., [24] and Christinal et al., [17] in chilli. Similiraly, Lo et al., [25] screened *Trichoderma* and found that influence of different strains on the growth of bitter gourd, loofah, and cucumber plants.

TABLE 2

Impact of *Trichoderma* isolates on radical length (cm) of chilli varieties

Treatment	Varieties				
	Pusa jwala	Kashmiri long	Pusa sadabahar	UHFC 12-4	Mean
TRNU-1	4.25	5.1	4.313	4.953	4.654
TRNU-2	4.823	4.37	4.303	4.778	4.568
TRNU-3	4.125	3.88	4.168	4.635	4.202
Control	3.953	3.408	3.453	3.55	3.591
Mean	4.29	4.189	4.06	4.479	
	Variety	Treatment	Variety × Treatment		
SE ±	0.071	0.071	0.142		
CD (1%)	0.143	0.143	0.287		

The mean plumule length ranged from 4.73 to 3.94 across the genotypes with significant difference (Table 3). The maximum plumule length among mean value of chilli varieties was observed in the case of UHFC 12-4 (4.70) followed by Kashmiri Long (4.69), Pusa Jwala (4.42), whereas the minimum plumule lenght was recorded in Pusa Sadabahar (4.09). Among all the treatments highest mean value of plumule length was found in TRNU-2 (4.874) and lowest was recorded in control (3.70). The interaction due to variety and treatments had significant difference for plumule length. Comparable range of variability for plumule length in chilli has also been reported by Dhanelappagol et al., [23], Hunje et al., [16] and Christinal et al., [17]. Methanol extract of *T. harzianum* and *T. viridi* significantly improved various growth parameters of okra [26]. Studies have been confirmed in case of *T. harzianum* and *T. viridi* to enhanced seed germination root [27] as well as increasing the frequency of healthy plants and boosting yield [28].

TABLE 3

Effect of *Trichoderma* isolate on plumule length of different chilli varieties

Treatment	Varieties				
	Pusa jwala	Kashmiri long	Pusa sadabahar	UHFC 12-4	Mean
TRNU-1	4.71	5.323	4.518	4.943	4.873
TRNU-2	4.223	5.67	4.775	4.828	4.874
TRNU-3	4.568	4.605	3.943	4.728	4.461
Control	4.185	3.178	3.14	4.31	3.703
Mean	4.421	4.694	4.094	4.702	
	Variety	Treatment	Variety × Treatment		
SE ±	0.051	0.051	0.101		
CD (1%)	0.102	0.102	0.205		

The mean seedling length ranged from 8.26 to 8.88 across the genotypes with significant difference (Table 4). The maximum seedling length among mean value of chilli varieties was observed in the case of Kashmiri Long (8.88) followed by UHFC 12-4 (8.693), Pusa Jwala (8.41), whereas the minimum seedling length was recorded in Pusa Sadabahar (8.26). Among all the treatments highest mean value of seedling length was found in TRNU-1 (9.147) and lowest was recorded in control (7.58). The interaction due to variety and treatments had significant difference for seedling length.

TABLE 4

Effect of *Trichoderma* isolate on seedling length of different chilli varieties

Treatment	Varieties				
	Pusa jwala	Kashmiri long	Pusa sadabahar	UHFC 12-4	Mean
TRNU-1	8.647	10.493	8.833	8.615	9.147
TRNU-2	8.923	8.81	8.968	9.588	9.072
TRNU-3	8.425	8.497	8.103	8.745	8.443
Control	7.65	7.728	7.148	7.823	7.587
Mean	8.411	8.882	8.263	8.693	
	Variety	Treatment	Variety × Treatment		
SE ±	0.033	0.033	0.065		
CD (1%)	0.066	0.066	0.132		

The mean 10 seedling fresh weight ranged from 0.41 to 0.43 across the genotypes with significant difference (Table 5). The maximum 10 seedling fresh weight among mean value of chilli varieties was observed in the case of Kashmiri Long (0.44) followed by Pusa Jawala (0.42), Pusa Sadabahar (0.42), whereas the minimum 10 seedling fresh weight was recorded in UHFC12-4 (0.41). Among all the treatments highest mean value of 10 seedlings fresh weight was found in TRNU-1 (0.47) and lowest was recorded in control (0.35). The interaction due to variety and treatments had significant difference for 10 seedlings fresh weight. Variation in seedling radical and plumule length of genotypes not only affected the overall vigour but also possessed a proportional relationship with the fresh weight of seedlings. The findings of present investigation could be correlated with that of Jolli et al., [29] who reported a range of fresh weight.

TABLE 5

Effect of *Trichoderma* isolate on seedling fresh weight of different chilli varieties

Treatment	Varieties				
	Pusa jwala	Kashmiri long	Pusa sadabahar	UHFC 12-4	Mean
TRNU-1	0.463	0.488	0.478	0.455	0.47
TRNU-2	0.45	0.428	0.408	0.423	0.43
TRNU-3	0.43	0.505	0.425	0.403	0.44
Control	0.338	0.325	0.355	0.38	0.35
Mean	0.42	0.44	0.42	0.41	
	Variety	Treatment	Variety × Treatment		
SE ±	0.023	0.023	0.047		
CD (1%)	N/A	0.047	N/A		

The mean 10 seedling dry weight ranged from 0.19 to 0.35 across the genotypes with significant difference (Table 6). The maximum 10 seedling fresh weight among mean value of chilli varieties was observed in the case of Pusa Jawala (0.35) followed by Pusa Sadabahar (0.21), UHFC 12-4(0.20), whereas the minimum 10 seedling fresh weight was recorded in Kashmiri Long (0.19). Among all the treatments highest mean value of 10 seedlings dry weight was found in TRNU-2 (0.26) and lowest was recorded in Control (0.21). The interaction due to variety and treatments had significant difference for 10 seedlings dry weight. There was a parallelism between 10 seedlings' dry weight and 10 seedlings' fresh weight indicating presence of seedling vigour due accumulation of dry matter in plant tissues. Variability in seedling dry weight of chilli genotypes has also been reported by Natesh et al., [15], Hunje et al., [16] and Christinal et al., [17].

TABLE 6

Effect of *Trichoderma* isolate on 10 seedlings dry weight of different chilli varieties

Treatments	Varieties				
	Pusa jwala	Kashmiri long	Pusa sadabahar	UHFC 12-4	Mean
TRNU-1	0.283	0.155	0.273	0.243	0.24
TRNU-2	0.675	0.12	0.125	0.135	0.26
TRNU-3	0.243	0.273	0.218	0.19	0.23
Control	0.2	0.2	0.215	0.22	0.21
Mean	0.35	0.19	0.21	0.2	
	Variety	Treatment	Variety × Treatment		
SE ±	0.03	0.03	0.059		
CD (1%)	0.06	N/A	0.119		

The mean value of vigour index-I ranged from 548.18 to 608.26 across the genotypes with significant difference (Table 7). The maximum vigour index-I among mean value of chilli varieties was observed in the case of Kashmiri Long (608.27) followed by Pusa Jwala (580.58), Pusa Sadabahar (512.92), whereas the minimum vigour index-I was recorded in UHFC 12-4(548.18). Among all the treatments highest mean value of vigour index-I was found in TRNU-2 (560.40) and lowest was recorded in control (537.71). The interaction due to variety and treatments had significant difference for vigour index-I. Germination percentage and seedling length were the majors

factor for deciding the vigour index-I. Variability in seedling vigour index-I in chilli genotypes have also been studied by Dhanelappagol et al., [23] who reported a wider range of variability however and Natesh et al., [15] in chilli. Similar findings have been documented by other researchers, indicating that seeds treated with inoculant extracts of *T. viride*, *T. harzianum* and *T. pseudokoningii* exhibited higher rates of seed germination, improved seedling vigor, and reduced occurrences of seed-borne fungal pathogens compared to the control group by Zheng et al., [30] and Bharath et al., [31].

TABLE 7

Effect of *Trichoderma* isolate on vigour index-I of different chilli varieties

Treatment	Varieties				
	Pusa Jwala	Kashmiri Long	Pusa Sadabahar	UHFC 12-4	Mean
TRNU-1	566.573	662.37	453.058	559.608	560.4
TRNU-2	629.458	526.9	512.44	618.41	571.8
TRNU-3	575.603	664.465	527.978	552.113	580.04
Control	550.67	579.343	558.223	462.598	537.71
Mean	580.58	608.27	512.924	548.182	
	Variety	Treatment	Variety × Treatment		
SE ±	5.948	5.948	11.895		
CD (1%)	11.996	11.996	23.991		

The mean value of vigour index-II ranged from 13.54 to 16.34 across the genotypes with significant difference (Table 8). The maximum vigour Index-II among mean value of chilli varieties was observed in the case of Pusa Jawala (16.34) followed by Kashmiri Long (14.97), Pusa Sadabahar (13.78), whereas the minimum vigour index-II was recorded in UHFC 12-4 (13.54). Among all the treatments highest mean value of vigour index-II was found in TRNU-1 (16.82) and lowest was recorded in control (11.52). The interaction due to variety and treatments had significant difference for vigour index-II. Seedling vigour index-II is helpful in monitoring and ensuring the survival and growth of seedlings after germination. This character showed significant variation, which might be due to genetic constitution of the genotypes. Corresponding results on variability in seedling vigour index-II in chilli genotypes have also been reported by Hunje et al., [16] and Kivadasannavar et al., [18]. Begum et al., [32] were evaluated five *Trichoderma* strains to assay their efficacy in suppressing *Alternaria* fruit rot disease of Chilli and promoting Chilli plant growth and yield and observed that application of *T. harzianum* IMI 392432 significantly suppressed the disease and improved highest seed germination percentage, vigour index, growth and yield.

TABLE 8

Effect of *Trichoderma* isolate on vigour index-II of different chilli varieties

Treatment	Varieties				
	Pusa jwala	Kashmiri long	Pusa sadabahar	UHFC 12-4	Mean
TRNU-1	18.695	16.715	16.725	15.165	16.825
TRNU-2	17.65	17.338	12.498	13.528	15.253
TRNU-3	15.413	15.593	14.125	15.055	15.046
Control	13.63	10.268	11.785	10.423	11.526
Mean	16.34	14.97	13.78	13.54	
	Variety	Treatment	Variety × Treatment		
SE ±	0.222	0.222	0.444		
CD (1%)	0.448	0.448	0.896		

CONCLUSION

The study observed significant effects of *Trichoderma* isolates on various parameters related to seed germination, seedling vigor, and growth in different varieties of Chilli. *Trichoderma* treatments, particularly TRNU-1 and TRNU-2, demonstrated notable enhancements in seed germination percentage, radical and plumule length, seedling length, as well as seedling fresh and dry weights. These findings suggest the potential of *Trichoderma* isolates in promoting Chilli plant growth and vigor. The results emphasize the significance of *Trichoderma* as a potential biocontrol agent and growth promoter in the cultivation of Chilli, offering promising prospects for sustainable agricultural practices and increased crop productivity.

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