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RESEARCH ARTICLE

ASSESSMENT OF *TRICHODERMA ASPERELLUM* ISOLATE MBCT10 FOR SCLEROTIUM FOOT AND ROOT ROT CONTROL IN LEGUME CROPS: LABORATORY AND FIELD EVALUATIONS

Sonia Naznin, Mohammad Shahjahan Monjil*

Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh
Correspondence Author Email: smonjil@bau.edu.bd

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ABSTRACT

Legume crops such as lentils, chickpeas, and soybeans are vital for human nutrition and soil fertility, particularly in regions like Bangladesh. However, the yield of these crops is frequently impacted by diseases, with foot and root rot, attributed to *Sclerotium rolfsii*, being a prominent concern. Conventional control measures have limitations, prompting the exploration of alternative strategies. This study evaluated the efficacy of *Trichoderma asperellum*, focusing on a native isolate MBCT10, in controlling foot and root rot in lentils, chickpeas, and soybeans. Laboratory experiments demonstrated MBCT10's ability to suppress the radial mycelial growth of *S. rolfsii*, confirmed through molecular characterization. Field experiments revealed that *T. asperellum* treatment significantly increased seed germination rates over control conditions for foot and root rot susceptible three legumes varieties, viz. Lentil var. Binamasur-6 by 6.50%, Chickpea var. Binachola-3 by 10.17%, and Soybean var. Binasoybean-2 by 64.79%, respectively. Additionally, *T. asperellum* decreased foot and root rot incidence compared to control at 60 days after sowing (DAS) by 26.42% for Binamasur-6, 49.80% for Binachola-3, and 34.84% for Binasoybean-2. Treated plots exhibited higher plant height, pods per plant, seed weight per plant, and dry plant weight per plant for all three crops. These findings underscore the potential of *T. asperellum* isolate MBCT10 as a sustainable and effective biocontrol agent for managing fungal diseases in legume crops, thereby promoting environmentally friendly agricultural practices.

KEYWORDS

Trichoderma asperellum Isolate MBCT10, Foot and root rot, Legume crops, Biocontrol agent, Disease incidence

1. INTRODUCTION

Legumes have long been recognized as crucial components of human nutrition, serving as significant sources of protein, essential vitamins, and minerals. Particularly in tropical and sub-tropical regions of Asia and Africa, legumes such as chickpeas, lentils, and soybeans play a vital role in providing affordable protein alternatives to millions of people (Sattar et al., 1996). Additionally, these legumes contribute to soil fertility through nitrogen fixation, forming symbiotic relationships with Rhizobia bacteria.

Among the varied selection of legumes, lentils (*Lens culinaris*), chickpeas (Cicer *arietinum*), and soybeans (*Glycine max*) are particularly noteworthy in Bangladesh due to their diverse applications. According to FAOSTAT, global production figures for lentils, chickpeas, and soybeans in 2016 were substantial, highlighting their significance in agricultural production worldwide (FAOSTAT, 2018).

In Bangladesh, however, the productivity of these legumes trails behind that of other countries, especially in comparison to Indian sub-continental countries. This productivity gap can be attributed to various production constraints, including biotic stresses, diseases, pests, seed dormancy, and weed infestations (Hoque et al., 2014). Among these constraints, diseases pose a significant threat to yield stability, with phytopathogenic fungi causing substantial losses from seedling to maturity stages (Hoque et al., 2014).

One of the most devastating diseases affecting legumes is foot and root rot, primarily caused by *Sclerotium rolfsii*. This fungal pathogen presents a significant danger to lentils, chickpeas, and soybeans, resulting in damping-off of seedlings, as well as stem and root rots, causing significant decreases in yield (Billah et al., 2017). With its ability to persist in soil for extended periods as sclerotia, *S. rolfsii* presents a formidable challenge to agricultural productivity.

Conventional control measures, such as chemical fungicides, have shown limited effectiveness against *S. rolfsii* due to soil heterogeneity and environmental concerns associated with chemical usage (Tewari and Mukhopadhyay, 2001). Furthermore, overdependence on chemical treatments has resulted in negative impacts on human health, environmental contamination, and the emergence of fungi resistant to fungicides (Rakh et al., 2011). Hence, there is a pressing need to explore alternative methods for disease management that are environmentally sustainable and less dependent on chemical inputs.

In recent years, biological control strategies have emerged as promising alternatives to chemical fungicides, offering eco-friendly solutions for disease management. Among these strategies, the use of antagonistic microorganisms, such as *Trichoderma* species, has gained traction due to their ability to suppress the proliferation of plant pathogens while promoting plant growth and nutrient uptake (Morang et al., 2013). *Trichoderma asperellum*, notably isolate MBCT10 with Accession No. OR125623, emerges as a significant biological control agent against

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various plant pathogens in Bangladesh. Derived from local sources, this strain presents a native solution to combat fungal adversaries (Khatun et al., 2021). Its adaptation to the region's environmental conditions underscores its efficacy in agricultural contexts specific to Bangladesh (Khan et al., 2020). By harnessing *T. asperellum* as a biopesticide, not only can agricultural practices become more eco-friendly, but also the delicate balance of beneficial microorganisms in the soil can be preserved (Harman, 2006). Field experiments have demonstrated its capacity to significantly reduce seedling disease incidence, leading to greater plant stands and enhanced yields (Mukherjee et al., 2013). Amidst a backdrop of increasing reliance on chemical pesticides, *T. asperellum* offers a sustainable alternative for safeguarding crop health while minimizing environmental disturbance.

In light of these considerations, this study aims to evaluate the efficacy of *T. asperellum* in controlling foot and root rot caused by *S. rolfsii* in chickpeas, lentils, and soybeans. Furthermore, the in vitro capability of *T. asperellum* to impede the radial mycelial growth of *S. rolfsii* will also be examined. Through these objectives, this research seeks to contribute to the development of sustainable and environmentally friendly approaches to combating fungal diseases in legume crops.

2. Materials And Methods

2.1 Laboratory experiment

2.1.1 Experimental setup and period

The isolation, morpho-molecular analysis, and bioassay of *T. asperellum* were carried out at the Microbiology & Biocontrol Laboratory in Bangladesh Agricultural University, Bangladesh, from January 20, 2019, to January 30, 2020. This laboratory experiment aimed to assess the antagonistic potential of a native isolate of *T. asperellum*, against *Sclerotium rolfsii*, which causes foot and root rot disease.

2.1.2 Isolation and characterization of Trichoderma

Soil samples were collected from various locations in Bangladesh to isolate *Trichoderma*. The samples were processed using standard dilution and plating techniques. *Trichoderma asperellum* colonies were identified based on their characteristic morphology on Potato Dextrose Agar (PDA) media. *T. asperellum* colonies were further characterized culturally and morphologically on PDA media. Specific isolates were selected for their distinct characteristics.

In an in-vitro study, multiple isolates of *T. asperellum* were tested for their antagonistic activities against soil and foliar pathogens. One isolate, MBCT10 of *T. asperellum*, exhibited significant antagonistic activity against both types of pathogens. The highly effective isolate, MBCT10, underwent detailed examination for colony color, type, growth characteristics, and conidia shape.

2.1.3 Molecular characterization

Molecular characterization of MBCT10 was performed using DNA extraction. The gene corresponding to the Internal Transcribed Spacer (ITS) region of MBCT10 was amplified using the ITS4/ITS5 primers (Martin and Rygiewicz, 2005). The obtained gene sequences were analyzed, and the results were deposited into the NCBI GeneBank.

2.1.4 Isolation and characterization of Sclerotium rolfsii

Isolation of foot rot pathogen of legume (lentil) was done through tissue culture technique. Infected root sections from lentil plants were meticulously excised using a sterile scalpel knife, then cut into 1–1.5 cm fragments, and subsequently sterilized by immersion in a 10% Chlorox solution for 1 minute. After sterilization, the root segments were thoroughly rinsed with sterile distilled water three times before being evenly spread onto Petri dishes containing PDA. The morphological characteristics of *Sclerotium rolfsii* were determined using established criteria outlined by Barnett and Hunter in 1972, which involved examining the features of mycelium and sclerotia. Additionally, the pathogenicity of *S. rolfsii* isolate was assessed through inoculation trials conducted on potted lentil, chickpea, and soybean plants, following the protocols outlined by (Billah et al., 2017).

2.1.5 Dual culture method for assessing antagonistic potential

The effectiveness of MBCT10 of *T. asperellum*, against *S. rolfsii* was assessed using the dual-culture technique (Dennis and Webster 1971) on potato dextrose agar (PDA) medium. and allowed to solidify. To examine the effect of *T. asperellum* on S. rolfsii, five-day-old cultures in PDA were used for both fungi. Initially, 20 ml of PDA was poured into petri dishes and left to solidify. Afterwards, mycelial discs (6 mm of diameter), sourced from the edges of 5-day-old cultures of both *T. asperellum* and *S. rolfsii*, were placed diametrically opposite each other on the plates, equidistant from the periphery, on the same times. Each treatment and control group comprised six replications. The experiment was meticulously conducted under sterile conditions, and all plates were placed in an incubator at 25 ± 1°C for 10 days. The growth of the targeted fungal pathogen's mycelium was assessed twice daily, at 9:00 AM and 5:00 PM, and the average of these assessments was computed. The inhibition percentage of the average radial growth of mycelial was determined by comparing it with the mycelial growth observed in the control plates, employing the formula: $I = [(C – T)/C] \times 100$ (Hajiegharai et al., 2008). Here, 'I' denotes the inhibition percentage, 'C' represents the radial growth measurement of the pathogen in the control, and 'T' signifies the radial growth of the pathogen in the presence of *T. asperellum*.

2.1.6 Data Analysis

The data obtained from the laboratory experiment were analyzed to calculate the mycelial growth inhibition of *S. rolfsii* by *T. asperellum*. The inhibition percentage was calculated by comparing the mycelial growth of *S. rolfsii* with and without the presence of *T. asperellum*.

2.2 Field Experiment

A field experiment was conducted to evaluate the effectiveness of the native isolate MBCT10 of *T. asperellum*, obtained from the laboratory experiment, in combating foot and root rot disease in lentil (Binamasur-6), chickpea (Binachola-3), and soybean (Binasoybean-2) crops. It was conducted at the research field of Bangladesh Institute of Nuclear Agriculture, located in Mymensingh, Bangladesh. The experiment spanned from November 2019 to April 2020, chosen to coincide with the growing season of the selected crops.

2.2.1 Land preparation and seed collection

Land preparation involved initial plowing to break the soil, followed by cross-plowing and laddering to achieve the desired soil tilth suitable for sowing the crops. The seeds of lentil, chickpea, and soybean were obtained from BINA, Mymensingh. These specific varieties were selected due to their susceptibility to *S. rolfsii* causing foot and root rot disease by, making them ideal candidates for evaluating the effectiveness of *T. asperellum*.

2.2.2 Seed treatment

Before sowing, the seeds were treated with a laboratory-formulated product containing *T. asperellum* (MBCT10), prepared with talcum powder as a carrier (1.5% WP), at a concentration of 2.5% of the seed weight. This treatment aimed to enhance the seeds' resistance against foot and root rot disease. The experiment comprised two treatments: T1, involving seeds treated with *T. asperellum*, and T2, serving as the control group with untreated seeds.

2.2.3 Plot establishment and sowing

The experimental plots were set up using a randomized block design, with three replications for each. Each plot measured 2.5 meters by 2 meters, with a spacing of 1 meter between plots and between replications. Treated seeds were sown at a depth of approximately 2.0 cm and immediately covered with soil. To maintain plot integrity and uniformity, weeding was performed twice at 25 and 40 days after sowing (DAS).

2.2.4 Disease monitoring and data collection

Throughout the experimental period, the plots were regularly monitored to observe the incidence and severity of foot and root rot disease. Plants displaying symptoms of the disease were identified and recorded. Additionally, data on various agronomic parameters, including germination rate, post-emergence mortality, disease incidence, plant height, pod yield, seed yield, and plant biomass, were collected. Ten randomly selected plants from each plot were uprooted carefully for data collection.

2.2.5 Disease incidence

The incidence of foot and root rot within the experimental plots was monitored during routine inspections at 30, 45, and 60 DAS. The percentage of disease incidence was determined by applying the formula: % Disease incidence = (Number of infected plants / Total number of plants) × 100%. The calculation of increase or decrease of various parameters over the control was facilitated by the application of the following formula:

Increase or decrease over control=(C–T/C)×100 wherein, T represents the values of different growth or disease parameters obtained from the application of *T. asperellum* , and C signifies the values of the same parameters derived from the untreated control group. This method enables a comparative analysis of the efficacy of *T. asperellum* in altering growth or disease-related factors when compared to untreated conditions.

2.2.6 Statistical analysis

The gathered data were tabulated and analyzed following randomized block design using three replications. Treatment means were compared using the Student t-test method to determine significant differences between treatments.

3. RESULTS

3.1 Laboratory Experiments

3.1.1 Morphological characteristics of MBCT10 of T. asperellum isolated from Native source of Bangladesh

Isolate MBCT10 of *T. asperellum* displayed distinctive morphological features when cultured on Potato Dextrose Agar (PDA) media. Initially, the colony appeared as circular, aerial, white mycelial mats resembling cotton with a smooth texture. By the 3rd to 4th day of cultivation, the colony's color transitioned to light green, ultimately turning dark green by the 7th day. This transition in coloration was observable across the entire 9cm Petri dish (figure 1). Globose conidia were observed upon microscopic examination of MBCT10. The conidia exhibited typical characteristics of *Trichoderma* spp., further confirming the identity of the isolate. DNA extraction, amplification of specific gene sequences, and subsequent sequencing were conducted to molecularly characterize MBCT10. The obtained gene sequences were deposited into the NCBI GeneBank under Accession No. OR125623. Analysis of the molecular data provided additional confirmation of MBCT10's identity as *T. asperellum*, contributing to its taxonomic classification and elucidating its genetic makeup.

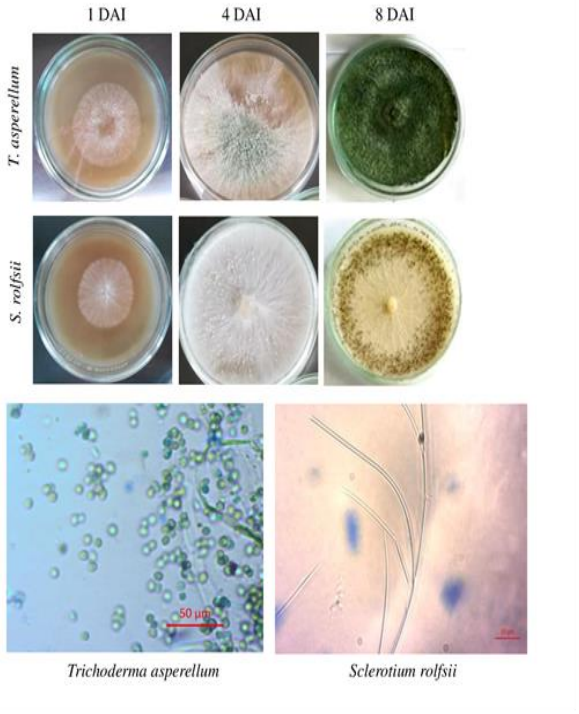

Figure 1: Mycelial growth of *Trichoderma asperellum* (MBCT10) and *Sclerotium rolfssii* on PDA

3.1.2 Morphological features in Sclerotium rolfsii

Sclerotium rolfsii was previously isolated from lentil field and, culturally and morphologically characterized on PDA. The fungal mycelium showed a silky white color, which later changed to a dull white with radial expansion. By the fourth day, as the fungus reached maturity, small mycelial knots began to develop, eventually transforming into sclerotia resembling mustard seeds by the seventh day of culturing. The resulting sclerotia exhibited deep brownish-black coloration, a shiny surface, and a hard texture, ranging in shape from spherical to irregular (Figure 1). Microscopic observation of the fungal culture revealed thin-walled, septate, hyaline aerial hyphae. The mycelium exhibited extensive branching and clamp connections. Pathogenecity test using lentil, chickpea and soybean produce foot and root rot symptoms.

3.1.3 Effect of T. asperellum in suppressing radial mycelial growth of S. rolfsii

The effect of MBCT10 from *T. asperellum* on inhibiting the radial mycelial growth of Scleoritum rolfsii was monitored over a duration of 10 days. By the 10th day, *T. asperellum* had completely inhibited *S. rolfsii* growth (Figure 2). On the third day of cultivation, the highest radial mycelial growth, reaching 9 cm, was noted for both *T. asperellum* and *S. rolfsii* in the control petri dishes. In contrast, the radial mycelial growth of both *T. asperellum* and *S. rolfsii* was slower in the dual-culture petri dishes. (Figure 2). By the third day of incubation, the radial mycelial growth of *T. asperellum* and *S. rolfsii* in the dual-culture Petri dish measured 4.29 cm and 4.35 cm, respectively. In contrast, in the control petri dishes, the growth reached its maximum of 9 cm on the third day. The peak radial mycelial growth (4.57 cm) of *S. rolfsii* in the dual-culture Petri dish was observed on the fifth day of incubation. Subsequently, the overgrowth of *T. asperellum* inhibited the mycelium of *S. rolfsii*. Eventually, by the 10th day, *T. asperellum* completely replaced the mycelial growth of *S. rolfsii*.

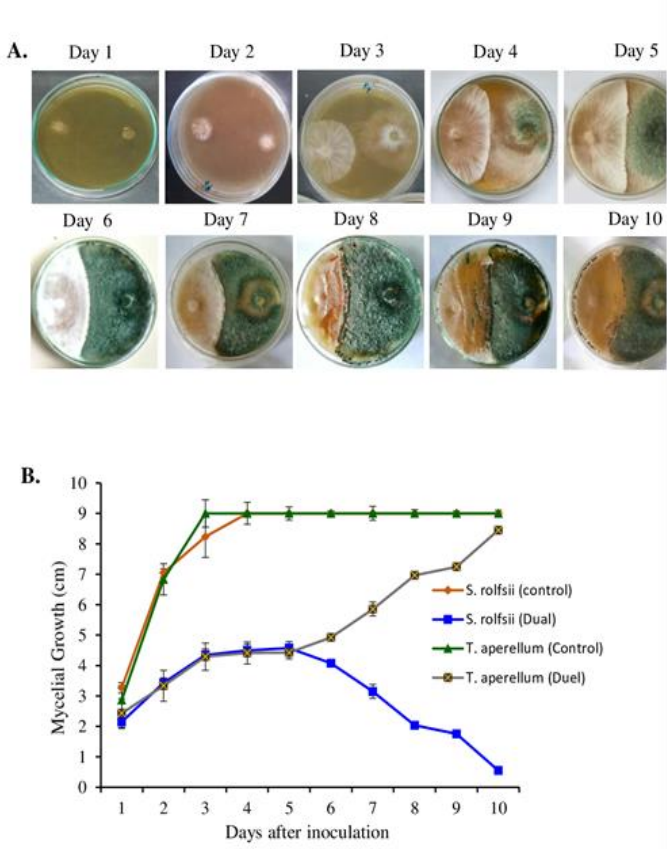

Figure 2: Mycelium growth of *Trichoderma asperellum* (MBCT10) and *Scelerotium rolfsii* in control and duel culture petridish, A. Dual culture on PDA media up to 10 days, and B. Quantification of mycelial growth in control and dual culture plates.

3.1.4 Effect of MBCT10 of T. asperellum on inhibition (%) of S. rolfsii in dual culture plate over control plate

The effect of MBCT10 of *T. asperellum* on inhibiting the growth of *S. rolfsii* in dual-culture plates, as compared to control plates, was investigated (Table 1). Over the course of 10 days, the efficacy of *T. asperellum* in inhibiting *S. rolfsii* was observed as follows: 34.18%, 51.39%, 51.70%, 50.00%, 49.19%, 54.70%, 65.04%, 77.41%, 80.52%, and 93.89% for the 1st through 10th days, respectively. The highest percent inhibition, 93.89%, was recorded on the 10th day. Conversely, the highest growth of S. rolfsii in the dual-culture plate, reaching 4.57 cm on the 5th day of incubation, corresponded to the lowest inhibition percentage (49.19%). However, subsequent to the fifth day of incubation, the mycelial growth of *S. rolfsii* in the dual-culture plate decreased due to the suppressive influence of *T. asperellum*, resulting in a simultaneous rise in inhibition percentage.

Table 1: Effect of *Trichoderma asperellum* on the Inhibition (%) of *S. rolfsii* in Dual Culture Plates Compared to Control Plates Over 10 Days.

Days	*S. rolfsii* (control)	*S. rolfsii* (Dual)	Inhibition (%) of *S. rolfsii* in duel culture over control
Day 1	3.27	2.15	34.18
Day 2	7.05	3.43	51.39
Day 3	9.00	4.35	51.70
Day 4	9.00	4.50	50.00
Day 5	9.00	4.57	49.19
Day 6	9.00	4.08	54.70
Day 7	9.00	3.15	65.04
Day 8	9.00	2.03	77.41
Day 9	9.00	1.75	80.52
Day 10	9.00	0.55	93.89

3.2 Field experiment

3.2.1 Effect of MBCT10 of T. asperellum on germination (%) of Binamasur-6, Binachola-3 and Binasoybean-2

Germination percentages were consistently higher in *T. asperellum* treated plots compared to control plots (Table 2). Specifically, the germination percentages of Binamasur-6 were 91.69% and 86.09% for *T. asperellum* treated and untreated plots, respectively. For Binachola-3, the percentages were 61.65% and 55.96% in *T. asperellum* treated and untreated plots, respectively. Similarly, for Binasoybean-2, the germination percentages were 68.52% and 41.58% in *T. asperellum* treated and untreated plots, respectively. The application of *T. asperellum* led to a significant increase in germination rates compared to the control, with improvements of 6.50%, 10.17%, and 64.79% recorded for Binamasur-6, Binachola-3, and Binasoybean-2, respectively.

Table 2: Effect of *T. asperellum* on germination (%) of Lentil var. Binamasur-6, Chickpea var. Binachola-3 and Soybean var. Binasoybean-2

Treatments	Germination %		
	Binamasur-6	Binachola-3	Binasoybean-2
T. asperellum	91.69	61.65	68.52
Control	86.09	55.96	41.58
Germination (%) increased over control	6.50	10.17	64.79
Level of Significance	*	*	**

Data were subjected to student *t-test* for *T. asperellum* and control treatments. Data presents the mean of three replications.

"+" indicates increase at the value (data) over control.

'*' and '**' indicate level of significance at 5% and 1%, respectively.

3.2.2 Effect of T. asperellum on disease incidence (%) of Binamasur-6, Binachola-3 and Binasoybean-2

The application of *T. asperellum* resulted in a significant reduction in disease incidence of foot and root rot across all three crops: Binamasur-6, Binachola-3, and Binasoybean-2 (Table 3). For Binamasur-6, the disease incidence was notably lower in plots treated with *T. asperellum* compared to the control at all observation intervals (30, 45, and 60 days after sowing [DAS]). Specifically, at 30 DAS, disease incidence decreased by 33.50% compared to the control, with a significant level of difference (*). Similar trends were observed at 45 DAS (-30.97%, *), and 60 DAS (-26.42%, **).

Table 3: Effect of *T. asperellum* on disease incidence (%) of Binamasur-6, Binachola-3 and Binasoybean-2

Crop name	% Disease incidence								
	Binamasur-6			Binachola-3			Binasoybean-2		
Treatments	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T. asperellum	12.92	16.92	22.03	8.97	11.56	17.29	25.41	26.83	34.41
Control	19.43	24.51	29.94	19.71	29.3	34.44	39.59	47.08	52.81
Decreased over control (%)	33.50	30.97	26.42	54.49	60.55	49.80	35.82	43.01	34.84
Level of Significance	*	*	**	*	*	*	**	**	*

Data were subjected to student *t-test* for *T. asperellum*and control treatments. Data presents the mean of three replications.

DAS=Days after sowing. "-" indicates decrease at the value (data) over control.

'*'and '**' indicate level of significance at 5% and 1%, respectively.

Similarly, for Binachola-3, *T. asperellum* application led to a substantial reduction in disease incidence compared to the control across all observation periods. The decrease in disease incidence was particularly pronounced at 45 DAS (-54.49%, *), followed by 60 DAS (-60.55%, *), and 30 DAS (-49.80%, *).

In the case of Binasoybean-2, *T. asperellum* treatment also resulted in significantly lower disease incidence compared to the control. The reductions were consistent across all observation intervals, with decreases of 35.82% (), 43.01% (), and 34.84% (**), at 30, 45, and 60 DAS, respectively. Overall, the application of *T. asperellum* demonstrated its effectiveness in reducing disease incidence in all three crops, providing promising prospects for disease management in agricultural practices.

3.2.3 Effect of *T. asperellum* on different growth and yield parameters of Binamasur-6, Binachola-3 and Binasoybean-2 during harvesting time

Effect of *T. asperellum* on plant height, total pod per plant, filled pod and unfilled pod per plant, seed weight and dry plant weight of Binamasur-6, Binachola-3 and Binasoybean-2 were evaluated and presented in Table 4. Plant height (cm) of Binamasur-6 was lower in control plots compared to *T. asperellum* treated plots. Highest plant height (cm) for Binamasur-6 was 29.88 cm recorder in *T. asperellum* treated plots and lowest was 26.8 cm recorded in untreated control plots. For Binamasur-6, highest number of pods per plant was 25.88 observed in *T. asperellum* treated plots in which filled pods and unfilled pods were 22.48 and 3.40, respectively and lowest number of pod per plant was 18 observed in untreated control plots in which filled pod and unfilled pod per plant were 9.3 and 8.7, respectively. Seed weight per plant in *T. asperellum* treated plots and untreated control plots were 3.56 g and 1.62 g, respectively. Highest and lowest dry plant weights were recorded for *T. asperellum* treated plots (27.67 g) and control plots (25g), respectively. Up to 11.49%, 43.78%, 119.75% and 10.68% higher plant height, pod per plant, seed weight per plant and dry plant weight per plant, respectively were recorded in *T. asperellum* treated plots over control.

Table 4: Effect of *T. asperellum* on different growth and yield parameters of Binamasur-6, Binachola-3 and Binasoybean-2 during harvesting time

Crop Name	Treatment	Plant height (cm)	Pod/ Plant	Filled Pod/ Plant	Unfilled Pod/ Plant	Seed Weight/Plant (g)	Dry plant weight/Plant (g)
Binamasur-6	*T. asperellum*	29.88	25.88	22.48	3.40	3.56	27.67
	Control	26.8	18	9.3	8.7	1.62	25
	Increased or decreased over control (%)	11.49	43.78	141.72	-60.92	119.75	10.68
	Level of Significance	*	**	**	**	*	*
Binachola-3	*T. asperellum*	57.73	48.2	43.4	4.80	33.03	85.3
	Control	50.1	18.14	8.87	9.27	12.13	72.76
	Increased or decreased over control (%)	15.23	165.71	389.29	-48.22	172.30	17.23
	Level of Significance	*	**	**	**	**	**
Binasoybean-2	*T. asperellum*	19.36	21.63	19.8	1.83	38.17	34.23
	Control	16.68	17.00	14.63	2.37	24.06	22.33
	Increased or decreased over control (%)	16.07	27.24	35.34	-22.78	58.65	53.29
	Level of Significance	*	**	**	*	*	**

Data were subjected to student *t-test* for *T. asperellum*and control treatments. Data presents the mean of three replications.

"+" indicates increase and "-" indicates decrease at the value (data) over control.

'*' and '**' indicate level of significance at 5% and 1%, respectively.

Plant height (cm) of Binachola-3 was lower in control plots compared to *T. asperellum* treated plots. Highest plant height (cm) for Binachola-3 was 57.73 cm recorder in *T. asperellum* treated plots and lowest was 50.1 cm recorded in untreated control plots. For Binachola-3, highest number of pods per plant was 48.2 observed in *T. asperellum* treated plots in which filled pods and unfilled pods were 43.4 and 4.80, respectively and lowest number of pod per plant was 18.14 observed in untreated control plots in which filled pod and unfilled pod per plant was 8.87 and 9.27, respectively. Seed weight per plant for *T. asperellum* treated plots and untreated control plots were 33.03 g and 12.13 g, respectively. Highest and lowest dry plant weights were recorded for *T. asperellum* treated plots (85.3 g) and control plots (72.76 g). Up to 15.23%, 165.71%, 172.30% and 17.23% higher plant height, pod per plant, seed weight per plant and dry plant weight per plant, respectively were recorded in *T. asperellum* treated plots over control.

Plant height (cm) of Binasoybean-2 was lower in control plots compared to *T. asperellum* treated plots. Highest plant height (cm) for Binasoybean-2 was 19.36 cm recorder in *T. asperellum* treated plots and lowest was 16.68 cm recorded in untreated control plots. For Binasoybean-2, highest number of pods per plant was 21.63 recorder in *T. asperellum* treated plots in which filled pods and unfilled pods were 19.8 and 1.83, respectively and lowest number of pod per plant was 17.00 recorded in untreated control plots in which filled pod and unfilled pod per plant was 14.63 and 2.37, respectively. Seed weight per plant for *T. asperellum* treated plots and untreated control plots were 38.17 g and 24.06 g, respectively. Highest and lowest dry plant weights were recorded in *T. asperellum* treated plots (34.23 g) and control plots (22.33 g). Up to 16.07%, 27.24%, 58.65% and 53.29% higher plant height, pod per plant, seed weight per plant and dry plant weight per plant, respectively were recorded in *T. asperellum* treated plots over control.

4. Discussion

The evaluation of *Trichoderma asperellum* isolate MBCT10 against foot and root rot in chickpeas, lentils, and soybeans caused by *Sclerotium rolfsii* presents promising insights into the potential of biocontrol agents in sustainable agriculture practices. *Trichoderma* species have long been recognized for their ability to act as effective biological control agents against various plant pathogens (Sousa and Blum, 2013). The efficacy of *T. asperellum* in mitigating diseases caused by *Sclerotium rolfsii*, a notorious fungal pathogen affecting legume crops, underscores its importance in disease management strategies. The present study builds upon previous research demonstrating the effectiveness of *Trichoderma* spp. in controlling diseases in legumes, including beans, peanuts, and soybeans (Kotasthane et al., 2015).

In the present research, improved seed germination rates was observed in *T. asperellum*-treated plots compared to control conditions. This improvement was particularly pronounced in lentil var. Binamasur-6, Chickpea var. Binachola-3, and Soybean var. Binasoybean-2, with germination rates increasing by up to 6.50%, 10.17%, and 64.79%, respectively. These results are consistent with studies by (Islam et al., 2016; Ashwini and Giri, 2014). Which highlight the positive impact of *Trichoderma* spp. on seed germination across various crops (Islam et al., 2016; Ashwini and Giri, 2014). Furthermore, field experiments revealed a substantial reduction in foot and root rot incidence in *T. asperellum*-treated plots compared to control conditions. This decrease in disease incidence is crucial for minimizing yield losses and ensuring the economic viability of legume cultivation. Similar findings have been reported in previous studies investigating the efficacy of *Trichoderma* spp. against foot and root rot diseases in lentils and other crops (Hannan et al., 2012; Gogoi et al., 2002). Study in 2014 found that soaking chickpea seeds in a conidial suspension of *Trichoderma* sp. for 30 minutes significantly increased seed germination (Ali et al., 2014).

In addition to disease control, compared to untreated plants, those treated with the *T. asperellum* exhibited better growth characteristics and yield parameters. These include increased plant height, pod number per plant, seed weight per plant, and dry plant weight per plant across all three crops. These enhancements in growth and yield are consistent with previous research highlighting the beneficial effects of *Trichoderma* spp. on crop performance (Hannan et al. 2012; Ahmed et al. 2009). This finding is consistent with the observations of researchers in 2021 who reported that soil application of *T. harzianum*-based BAU-biofungicide led to increased yield and improved all yield-contributing traits in wheat (Khan et al., 2021). Treating chickpea seeds with three species of *Trichoderma* - *T. harzianum*, *T. viride*, and *T. koningii* - resulted in enhanced plant growth (Amna et al., 2014).

Moreover, in the dual culture experiment on PDA, *T. asperellum* isolate MBCT10 exhibited the highest percent inhibition (93.89%) against *S. rolfsii* after 10 days. A study in 2014 similarly investigated the antagonistic potential of *T. harzianum* against *S. rolfsii* using the dual culture technique and found a percent inhibition of 76.76% (Yasmin et al., 2014). Additionally, The antagonistic fungus *T. harzianum* UD12-102 showed 90% inhibition against *S. rolfsii* in vitro and resulted in 80% survival of tomatoes infected by *S. rolfsii* in vivo (Suriyagamon et al., 2018). The bioagent Bio-arc (*T. albium*) was highly effective against S. rolfsii growth, with a 44.66% inhibition rate (Nawar, 2013). Furthermore, researchers evaluated nine antagonistic microorganisms using the dual culture technique for their effect against *S. rolfsii* under in vitro conditions, noting the maximum inhibition of mycelial growth (71.67%) with *T. asperellum* (Nagamma and Nagaraja, 2015). These findings collectively suggest that the application of these microorganisms successfully reduces root rot incidence and promotes the growth of chickpea plants.

5. Conclusion

The study evaluates *Trichoderma asperellum* isolate MBCT10 for controlling foot and root rot in lentils, chickpeas, and soybeans. Laboratory tests confirm its antagonistic activity against *Sclerotium rolfsii*. Field experiments demonstrate significant increases in seed germination and reductions in disease incidence with MBCT10 treatment. Moreover, treated plots exhibit improved plant growth and yield parameters. The

dual culture technique shows MBCT10's high inhibition percentage (93.89%) against *S. rolfsii*. These results confirm the potential of *T. asperellum* MBCT10 as a sustainable biocontrol agent for managing fungal diseases in legume crops, promoting eco-friendly agricultural practices.

Author Contribution

S.N., designed the research, collected and analyzed data, and wrote the manuscript. H.I., and M.S.M. supervised the research throughout all the processes.

Conflict of Interest

The authors declare that there is no conflict of interest in preparing this research article.

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