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Enhancing Biocontrol Potential: Development and Efficacy Assessment of a Liquid Formulation of *Trichoderma Asperellum* MSU007 against *Sclerotium Rrolfsii*

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Abstract

Global agricultural systems face significant challenges due to the devastating impact of *Sclerotium rolfsii*, a plant pathogen responsible for substantial crop yield losses. Considering this problem, this study aims to enhance biocontrol potential against *S. rolfsii* by developing and evaluating a liquid formulation of *Trichoderma asperellum* MSU007. We prepared 4 distinct liquid formulations of *T. asperellum* MSU007, incorporating various protectants: 10 % molasses, 5 % molasses, 5 % lactose and 5 % trehalose. After 45 days at 28 ± 2 °C, *T. asperellum* MSU007 spore counts remained consistent at 10^7 spores/mL across all formulations. All formulations resulted in no growth and effectively suppressed *S. rolfsii* mycelium growth at concentration of 4, 5 and 6 mL/L. Transferring inhibited mycelium to fresh Potato Dextrose Agar (PDA) showed growth resembling *T. asperellum* MSU007 within 48 h. Terrachor Super-X fungicide fully suppressed growth. Notably, the 5 % trehalose formulation continued inhibiting sclerotia germination even after 45-day storage. This study highlights *T. asperellum* MSU007 formulations, particularly those based on trehalose as promising agents for disease control and prolonged storage stability. Future mechanistic investigations will be crucial to comprehensive understanding and application.

Keywords: Biocontrol, *Trichoderma asperellum* MSU007, Liquid formulation, Protectants, Sclerotium wilt, Sustainable agriculture

Introduction

Sclerotium rolfsii is a formidable plant pathogenic fungus, inflicting substantial losses across global crop production. Presently, the management of *S. rolfsii* predominantly relies on synthetic fungicides, which engender detrimental consequences for both the environment and human well-being. Consequently, it is imperative to forge efficacious and sustainable alternatives for combatting this pervasive pathogen. Several *Trichoderma* isolates have been investigated for their potential in mitigating sclerotium wilt, with selected isolates demonstrating efficacy in reducing sclerotial germination [1].

Biological control of plant diseases using *Trichoderma* species is an eco-friendly approach. Trichoderma, a soil and plant-root-dwelling fungus, is known for its ability to against plant pathogens through competition, mycoparasitism and the production of inhibitory compounds. Additionally, it enhances plant defenses, reducing the need for chemical pesticides and promoting sustainable agriculture [2]. Among these alternatives, *Trichoderma asperellum* emerges as a well-explored biocontrol fungus, lauded for its antagonistic potency against a various of plant pathogens. The biocontrol attributes of *T. asperellum* encompass diverse mechanisms, including nutrient competition, antibiosis, and mycoparasitism. Notably, it orchestrates its control measures through the secretion of cell wall-degrading enzymes and the synthesis of antifungal metabolites [3]. However, the effective deployment of *T. asperellum* against *S. rolfsii* confronts inherent challenges. A crucial limitation is the absence of a convenient and efficient formulation amenable to field application. Predominantly available in solid granules or powder formats, extant formulations pose challenges in terms of handling and uniform application. The impetus driving this research is to augment the biocontrol prowess of *T. asperellum* against *S. rolfsii* through the development and evaluation of a liquid formulation. The transition to a liquid formulation promises manifold advantages over traditional solid counterparts. Firstly, liquid formulations can be seamlessly blended with water and dispensed *via* spray, ensuring even distribution and comprehensive coverage. This, in turn, fosters heightened interaction between the biocontrol agent and the

pathogen, culminating in maximized efficacy [4,5]. Secondly, liquid formulations benefit from extended shelf life and heightened stability in contrast to their solid counterparts, thereby ameliorating the risk of viability deterioration and preserving product consistency [6,7]. Expanding on the research by Kolombet *et al.* [8], a long-lasting liquid formulation of the biofungicide *Trichoderma asperellum* was developed. This strain effectively controls Fusarium head blight. Key enhancements included starch as a food source, pH adjustment to reduce metabolic activity, and minimal copper addition. Maintaining oxygen availability was vital. The optimized formulation remains active for at least 6 months at room temperature. In addition, Oros and Naár [9] confirmed that *Trichoderma*-based mycofungicides are efficient in managing foliar diseases, improving crop quality, and reducing yield losses. Liquid formulations require less *Trichoderma* than solid ones. Sensitivity to *Trichoderma*-produced toxins varied among 13 phytopathogenic fungi, with *Pythium irregulare* being most sensitive and *Phytophthora infestans* and *Macrophomina phaseolina* less so. It was also noted that *Trichoderma* strains suspended in pharmaceutical-grade liquid paraffin survived for over 2 years, while emulsifiers in commercial liquid paraffin reduced their shelf life and made it toxic when used as a leaf spray. Furthermore, their easy integration into established agricultural practices makes liquid formulations more accessible and practical for farmers to adopt [10].

By innovating a liquid formulation for *T. asperellum*, the research aims to surmount the challenges posed by extant formulations, thereby delivering a pragmatic and efficacious tool for *S. rolfsii* management. The potency of the developed formulation will be subjected to rigorous experimentation, assessing its competence in *S. rolfsii* control and compatibility with prevailing agricultural practices. Essentially, this study aims to enhance sustainable agriculture by offering an environmentally friendly alternative to synthetic fungicides for controlling *S. rolfsii*. This approach seeks to reduce environmental impacts and promote the use of biocontrol agents in plant disease management.

Materials and methods

Microorganisms

Trichoderma asperellum MSU007 with antagonistic properties was sourced from soil in a durian field located in Sisaket province in northeastern Thailand [11]. Meanwhile, *Sclerotium rolfsii* was obtained from the Microbiology Laboratory within the Department of Biology at the Faculty of Science, Mahasarakham University, Thailand.

Evaluation of *Trichoderma asperellum* MSU007 efficacy against *Sclerotium rolfsii* using dual culture technique

The efficacy assessment of *T. asperellum* MSU007 in antagonizing *S. rolfsii* was conducted through a dual culture technique. Prior to experimentation, *T. asperellum* MSU007 was cultivated on Potato Dextrose Agar (PDA) plates for 7 days, while *S. rolfsii* was cultured on PDA plates for 4 days. An 8-mm diameter agar disc infused with *T. asperellum* MSU007 mycelium was placed 2 cm distant from the edge of a Petri dish. Simultaneously, an agar disc of identical dimensions and harboring *S. rolfsii* was positioned at a similar distance from the opposing edge of the Petri dish, effectively creating a dual culture setup. For comparative analysis, *S. rolfsii* was placed under analogous conditions on a control PDA plate. The experimental procedure was replicated thrice, and incubation was conducted at a room temperature (28 ± 2 °C). Antagonistic activity was gauged after a 7-day incubation period. Evaluation involved measuring the radius of the *S. rolfsii* colony in proximity to the *T. asperellum* MSU007 colony's direction, as well as the radius of the *S. rolfsii* colony on the control plate. The data was employed to compute the Percentage Inhibition of Radius Growth (PIRG) and Percentage Mycelium Overgrowth (POG) of the pathogenic fungus, as per the formulation [12,13].

The Percentage Inhibition of Radius Growth (PIRG) was calculated using the following formula: PIRG = (R1 - R2) / R1×100. Where: R1 signifies the mean radius of the *S. rolfsii* colonies thriving on agar plates in the control treatment. R2 denotes the mean radius of the *S. rolfsii* colonies flourishing on agar plates in the test method.

Similarly, the Percentage Mycelium Overgrowth (POG) was determined through the formula: POG = (C1 - C2) / D×100. Where: C1 represents the mean radius of the *T. asperellum* MSU007 mycelium encompassing the S. *rolfsii* mycelium on the assessment day. C2 signifies the mean radius of the *T. asperellum* MSU007 mycelium encompassing the *S. rolfsii* mycelium before the commencement of the study. D denotes the study duration.

Development of liquid formulation of *T. asperellum* MSU007

To develop a liquid formulation for *T. asperellum* MSU007, 4 distinct formulations were prepared. The ingredients for each formulation were thoroughly mixed, followed by autoclaving at 121 °C for 15 min, and subsequent cooling. *Trichoderma* spore suspensions were prepared by cultivating them on PDA medium for 7 days. Subsequently, the spores were collected and transferred into sterile distilled water, adjusted to a concentration of 10^8 cfu/mL, and introduced into each formulation, ensuring proper mixing before storage at room temperature, away from light. The components of each formulation are detailed in **Table 1**.

Table 1 Components of liquid formulation.

Components	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Molasses (mL)	100	50	-	-
Lactose (g)	-	-	50	-
Trehalose (g)	-	-	-	50
Tween 80 (mL)	30	30	30	30
KCl (g)	50	50	50	50
T.asperellum MSU007 (mL)	100	100	100	100
dH_2O (mL)	720	770	770	770
Total volume (mL)	1,000	1,000	1,000	1,000

Viability assessment of *T. asperellum* MSU007 in formulation

To assess the viability of *T. asperellum* MSU007 within the developed formulations, a series of experiments were conducted. Each of the 4 formulations was appropriately diluted, and the viability of *T. asperellum* MSU007 was evaluated utilizing the spread plate technique. The spread plate technique involved the application of diluted samples onto Rose bengal agar medium. Subsequently, the plates were incubated at room temperature (28 ± 2 °C) for a duration of 24 - 48 h, allowing the fungal colonies to develop. Viability assessments were performed by quantifying the colonies of *T. asperellum* MSU007 present on the plates.

The viability tests were conducted at multiple time intervals, specifically at 15, 30 and 45 days after the initiation of storage. This extended duration allowed for an assessment of the longevity of viability within the formulations. The experimental setup included a total of 3 replicates, ensuring reliable and representative results.

Assessment *of T. asperellum* MSU007 liquid formulation efficacy against *S. rolfsii* mycelium growth

The efficacy of the *T. asperellum* MSU007 liquid formulation in suppressing *S. rolfsii* mycelium growth was systematically evaluated. *S. rolfsii* mycelium was cultivated on PDA plates and subjected to a 7-day incubation period at room temperature (28 ± 2 °C). Circular mycelial samples, 8 mm in diameter, were meticulously excised using a cork borer. Subsequently, 5 of these samples were placed into sterile Petri plates, each containing 20 mL of the meticulously prepared *T. asperellum* MSU007 liquid formulation. The Petri plates were then incubated under room temperature conditions, and the experimental results were meticulously documented.

This study included 3 replications, with assessments conducted at 15, 30 and 45-day intervals. The liquid formulation was administered at varying concentrations is 4, 5 and 6 mL/1 L of water. Following a 72-hour incubation period, mycelium growth observations were made for all 4 formulations. A scoring system involving 3 tiers was employed: 0 signified an absence of mycelium growth surrounding the agar disc, + indicated mycelium growth encircling the agar disc, and ++ indicated a substantial mycelium growth around the agar disc. Thereafter, the agar discs hosting the mycelial samples were transposed onto PDA medium. Observations were recorded after an additional 48 h incubation period, encompassing the characteristics of both *S. rolfsii* and *T. asperellum* MSU007 mycelia.

Evaluation of *T. asperellum* MSU007 liquid formulation efficacy in Inhibiting sclerotia germination

The effectiveness of the *T. asperellum* MSU007 liquid formulation in restraining the germination o*f S. rolfsii* sclerotia was rigorously examined through a series of controlled experiments. The cultivation of *S. rolfsii* was performed on PDA plates and subsequent incubation at room temperature (28 ± 2 °C) for 14 days. After the stipulated incubation period, the sclerotia were acclimatized to room temperature for further manipulation, and the resulting experimental data were recorded.

The study involved 3 replications, and assessments were conducted at 15, 30, and 45 days. The liquid formulation was administered at different concentrations of 4, 5 and 6 mL/1 L of water. After a 72-hour incubation period, mycelium growth observations were made for all 4 formulations. A scoring system with 3 scale was used: 0 represented the absence of mycelium growth around the sclerotia, + indicated mycelium growth encircling the sclerotia, and ++ denoted substantial mycelium growth around the sclerotia. Subsequently, the sclerotia containing the mycelial samples were transferred to PDA medium, and observations of the characteristics of both *S. rolfsii* and *T. asperellum* MSU007 mycelia were recorded after an additional 48 h incubation period.

Results and discussion

Evaluation of *Trichoderma* asperellum MSU007 efficacy against *Sclerotium rolfsii* using dual culture technique

The antagonism of *T. asperellum* MSU007 against *S. rolfsii* was tested by dual culture technique 7 days after inoculation. The results showed that *T. asperellum* MSU007 inhibited mycelium growth and overgrowth of *S. rolfsii* was 54.16 and 2.5 %, respectively. The evaluation of *T. asperellum* MSU007 effectiveness against *S. rolfsii* was conducted through the dual culture technique after a 7-day inoculation period. This technique facilitates the examination of antagonistic interactions between the 2 organisms. The findings of the assessment revealed notable outcomes. *T. asperellum* MSU007 exhibited the capability to impede the mycelium growth of *S. rolfsii* by 54.16 %. Conversely, *T. asperellum* MSU007 exhibited a marginal overgrowth of 2.5 % in relation to the mycelium of *S. rolfsii.* These observations underscore the potential antagonistic role of *T. asperellum* MSU007 against *S. rolfsii*, providing valuable insights into its potential utility in mitigating the growth of this pathogenic fungus. This finding is consistent with previous research highlighting the biocontrol potential of *Trichoderma* species against various plant pathogens [1]. In a similar study, Silva *et al.* [14] investigated the biocontrol capabilities of indigenous Brazilian *Trichoderma* strains, *T. asperelloides* CMAA 1584 and *T. lentiforme* CMAA 1585, against *Sclerotinia sclerotiorum*. These strains were found to release volatile organic compounds inhibiting mycelial growth and sclerotia formation in *S. sclerotiorum*. Moreover, they demonstrated mycoparasitic activity on sclerotia, further emphasizing the biocontrol potential of *Trichoderm*a species.

Development of liquid formulation of *T. asperellum* MSU007

This section of the study pertains to the development of 4 distinct liquid formulations of *T. asperellum* MSU007, with ensuing observations as follows.

Formulation 1

The formulation exhibited a dark brown coloration. Upon being stored at room temperature (28 ± 2 °C), the spores of *T. asperellum* MSU007 were observed to settle at the glass bottom of the container. After a 15-day storage, the formulation underwent gelatinous agglomeration, with *T. asperellum* MSU007 spores forming white mycelium on the formulation surface. Subsequent storage durations of 30 and 45 days exhibited the development of dark green spores from the mycelium on the matured formulation.

Formulation 2

Similar to the previous formulation, the initial state of this formulation was characterized by a dark brown hue. The spores of *T. asperellum* MSU007 also precipitated to the container bottom when stored at room temperature (28 ± 2 °C). After 15 days, the formulation formed a gelatinous conglomerate, accompanied by white mycelium growth of *T. asperellum* MSU007 on the surface. Storage for 30 and 45 days resulted in the emergence of dark green spores from the matured formulation's mycelium.

Formulation 3

This formulation had a dark green appearance. Room temperature (28 ± 2 °C) storage led to the precipitation of *T. asperellum* MSU007 spores to the container bottom. The final formulation color was

characterized as opaque white. Throughout storage at 15, 30 and 45 days, the formulation exhibited a jelly-like agglomeration.

Formulation 4

As with Formulation 3, the visual characteristic of this formulation was dark green. Storage at room temperature caused *T. asperellum* MSU007 spores to precipitate to the container base. The final formulation color was approximately of opaque white. Over the course of storage for 15, 30 and 45 days, the formulation demonstrated agglomeration akin to suspended jelly-like materials mentioned above.

These empirical findings contribute insight into the dynamic transformations and appearances of the formulated liquid preparations of *T. asperellum* MSU007 over various storage periods.

Viability assessment of *T. asperellum* MSU007 in formulation

The evaluation of *T. asperellum* MSU007 viability within the formulated solutions revealed negligible differences across the 4 distinct formulations. Viability examinations were conducted at intervals of 15, 30, and 45 days (**Table 2**) and the results are presented below. Upon the 15 days of storage of the formulations, the recorded viability of *T. asperellum* MSU007 stood at 2.4×10^6, 5.1×10^7, 4.8×10^7 and 9.3×10^7 cfu/mL within formulation 1, 2, 3 and 4, respectively. After 30 days of storage, *T. asperellum* MSU007 viability within the formulations was 1.0×10^6, 1.5×10^7, 1.8×10^7 and 5.6×10^7 cfu/mL within formulation 1, 2, 3 and 4, respectively. Upon reaching the 45-day threshold of formulation storage, *T. asperellum* MSU007 viability within the formulations was 1.1×10^4, 1.1×10^7, 1.7×10^7 and 1.0×10^7 cfu/mL within formulation 1, 2, 3 and 4, respectively. These findings underscore the consistent viability maintenance of *T. asperellum* MSU007 across the distinct formulations, elucidating its longevity within the liquid preparations. To enhance the practical application of *T. asperellum* MSU007, 4 distinct liquid formulations were developed, incorporating the protective agents molasses, lactose and trehalose. Notably, the 5 % trehalose formulation retained its ability to inhibit sclerotia germination even after 45 days of storage. The decline in viability of T. asperellum MSU007 in Formulation 4 after 45 days compared to 30 days is likely due to the diminishing effectiveness of trehalose as a stabilizer over extended storage. Changes in microbial activity within the formulation and variations in storage conditions like temperature and humidity could have also played a role. Liu *et al.* [15] similarly improved the stability of antagonist yeast strains using lactose and trehalose, highlighting the importance of trehalose in enhancing the stability and efficacy of liquid formulations. The viability of *T. asperellum* MSU007 within the liquid formulations was consistent over the 45-day storage period, demonstrating their suitability for prolonged storage, a critical factor for agricultural applications [16]. In contrast to prior research by Rezende *et al.* [17], which primarily focused on commercial *Trichoderma* products derived from solid-state fermentation, the study emphasized the advantages of submerged liquid fermentation for large-scale production of *Trichoderma* spp. They aimed to optimize key variables affecting the liquid fermentation process of *Trichoderma asperelloides* LQC-96 to enhance conidial production and its effectiveness against *S. sclerotiorum*. The highest conidial concentration was achieved after a 7-day fermentation period. Under these optimized conditions, they also assessed the cultivation of *T. erinaceum* T-12, T-18 and *T. harzianum* T-15. However, only *T. asperelloides* LQC-96 demonstrated efficient parasitism of *S. sclerotiorum*, preventing sclerotium myceliogenic germination. These findings allow the proposal of a set of optimal fermentation conditions that maximize conidial production of *T. asperelloides*, positioning as a promising biofungicide against *S. sclerotiorum*.

Table 2 Viability a of *T. asperellum* MSU007 within formulation.

Storage (day)	Viability of *T. asperellum* MSU007 (cfu/mL)			
	Formulation 1	**Formulation 2**	**Formulation 3**	**Formulation 4**
15	$2.4 \pm 0.24\times10^6$	$5.1 \pm 0.24\times10^7$	$4.8 \pm 0.24\times10^7$	$9.3 \pm 0.49\times10^7$
30	$1.0 \pm 0.24\times10^6$	$1.5 \pm 0.33\times10^7$	$1.8 \pm 0.49\times10^7$	$5.6 \pm 0.49\times10^7$
45	$1.1 \pm 0.16\times10^4$	$1.1 \pm 0.24\times10^7$	$1.7 \pm 0.41\times10^7$	$1.0 \pm 0.16\times10^7$

Assessment *of T. asperellum* MSU007 liquid formulation efficacy against *S. rolfsii* mycelium growth

The primary objective of this investigation was to assess the formulation performance in inhibiting the hyphal growth of *S. rolfsii* across diverse application rates, specifically at concentrations are 4, 5 and 6 mL/1 L of water. It is noteworthy that all formulations consistently demonstrated efficacy in restraining the

expansion of *S. rolfsii* hyphae, regardless of the application rate. Subsequently, inhibition of mycelium growth was elucidated within all 4 formulations, with the outcomes recorded at 72 h after incubation. This assessment employed a scoring system representing mycelial proliferation. Importantly, all 4 formulations exhibited effective suppression of mycelium growth at each application rate, regardless of the storage duration (15, 30 and 45 days). In all instances, a score of 0 was assigned, signifying the absence of mycelial growth surrounding the agar disc (**Figure 1**).

Further investigations involved the transfer of the agar disc onto fresh PDA plates, followed by an incubation period of 48 h. Remarkably, the ensuing hyphal growth displayed discernible characteristics, manifesting as white and fluffy structures, reminiscent of the hyphae of *T. asperellum* MSU007. This observation indicates that the mycelial growth of *S. rolfsii* was effectively suppressed by *T. asperellum* MSU007. Conversely, it was observed that *T. asperellum* MSU007 hyphae exhibited growth under these conditions. In addition to the formulation, the efficacy of a chemical control agent, Terrrachor Super-X was assessed in inhibiting mycelium growth. The results revealed that Terrrachor Super-X also received a score of 0, affirming its efficacy in restraining mycelium growth. Subsequently, when the agar disc treated with Terrrachor Super-X was transferred to fresh PDA plates, no discernible mycelium growth was observed. These findings collectively underscore the consistent and robust inhibitory effect of both the *T. asperellum* MSU007 liquid formulation and Terrrachor Super-X against *S. rolfsii* mycelium growth, even after extended storage duration. Chowdhury *et al.* [18] explored the potential of using microencapsulated *T. harzianum* in combination with organic additives as a biocontrol strategy against *S. rolfsii,* with the dual aim of improving both the overall health and yield of potato crops. Their investigation involved 5 distinct treatment combinations, including a control group with no *T. harzianum* or organic additives, and various combinations of *T. harzianum* with rice husk biochar, mustard oil cake, and tea waste. Before initiating the polyhouse experiments, the researchers evaluated the hyper-parasitic potential of *T. harzianum* against *S. rolfsii in vitro*, resulting in an impressive 63.2 % inhibition of *S. rolfsii* growth. Subsequently, the polyhouse study revealed significant improvements across multiple parameters, such as increased plant height, branch number per plant, leaf number per plant, shoot dry matter, root dry matter, healthy tuber formation, total tuber yield, leaf greenness, net photosynthetic rate, total phenolic content, and total antioxidant activity. Additionally, the treatment involving *T. harzianum* and organic additives led to a substantial reduction of 49 % in unhealthy tubers and a remarkable 32 % decrease in the disease severity index. These findings contribute to the development of effective and sustainable strategies for protecting potato crops from this damaging pathogen.

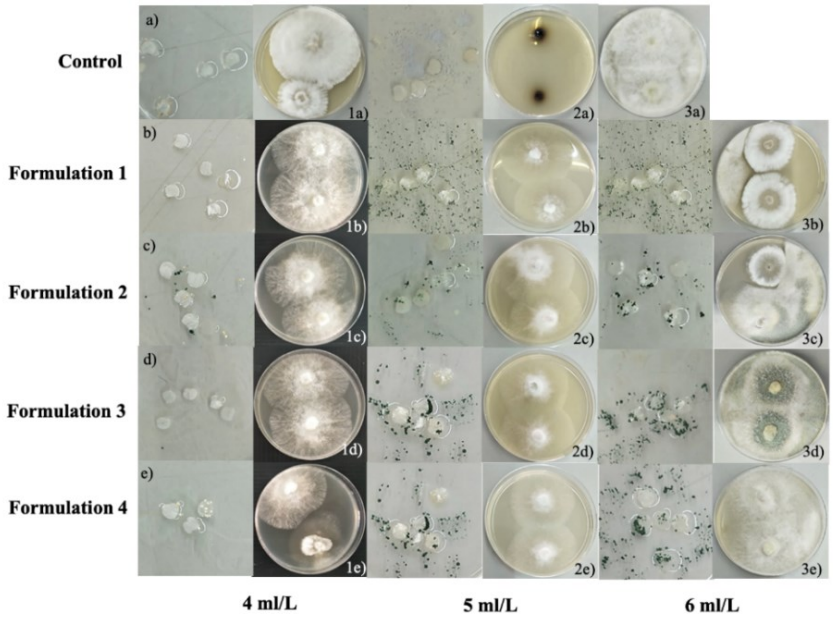

Figure 1 Efficacy of *T. asperellum* MSU007 liquid formulation in inhibiting the growth of *S. rolfsii* mycelium. On the left side of each plate, the inhibition of mycelium growth in the formulation is shown. On the right side, the colony morphology is displayed after transfer to a new PDA plate. (a) Control: (1a) dH2O, (2a) Terrachor Super-X, (3a) *Trichoderma asperellum* MSU 007, (b) Formulation 1, (c) Formulation 2, (d) Formulation 3, (e) Formulation 4.

Evaluation of *T. asperellum* MSU007 liquid formulation efficacy in inhibiting sclerotia germination

In the experiment, we evaluated the effectiveness of different *Trichoderma* formulations in inhibiting the germination of *S. rofsii* sclerotia over various storage times. The findings revealed the following (**Table 3**, **Figure 2**).

Formulation 1

This formulation demonstrated concentration-dependent mycelial growth inhibition. At 4 mL/L, there was substantial mycelial growth at all observed time points. At 5 mL/L, initial mycelial growth was minimal but increased over time. Remarkably, at 6 mL/L, complete inhibition of mycelial growth was achieved by day 45.

Formulation 2

Formulation 2 exhibited variable mycelial growth inhibition. At 4 mL/L, mycelial growth was initially noticeable but diminished by day 45. At 5 mL/L, limited mycelial growth was initially observed and significantly increased by day 45. At 6 mL/L, limited mycelial growth was noted initially, with a slight increase by day 45. The most effective inhibition was observed at 4 mL/L by day 45.

Formulation 3

This formulation displayed varying degrees of mycelial growth inhibition. At 4 mL/L, there was initial mycelial growth, which increased slightly by day 30 and became more pronounced by day 45. At 5 mL/L, limited mycelial growth was observed initially, with slight increases noted by day 45. At 6 mL/L, mycelial growth was limited, and no additional growth was observed by day 45. The most effective inhibition was at 6 mL/L by day 45.

Formulation 4

Formulation 4 consistently and effectively inhibited mycelial growth at all concentrations and time points. At 4 and 5 mL/L, no mycelial growth was observed at any time point. At 6 mL/L, limited mycelial growth was initially noted but did not increase further by day 30 or 45.

Terrrachor Super-X consistently inhibited mycelial growth effectively at a concentration of 3 mL/L throughout the experiment, with no mycelial growth observed at any of the 3 time points (15, 30 and 45 days). In the control group (dH_2O), without *Trichoderma*, extensive and persistent mycelial growth was observed at all time points. The mycelial growth not only persisted but also continued to increase, remaining extensive throughout the experiment.

It can be concluded that formulation 4 and Terrrachor Super-X were the most effective in inhibiting sclerotia germination, with formulation 4 being notably effective at lower concentrations. The control group demonstrated significant mycelial growth, emphasizing the efficacy of these formulations in suppressing *S. rofsii* sclerotia germination.

When sclerotia from each treatment were transferred onto fresh PDA plates and subsequently incubated at 28 ± 2 °C for 48 h. In the treatment utilizing the formulations, the emerging hyphae from the sclerotia exhibited characteristics resembling those of white, fluffy, rapidly proliferating hyphae akin to those typically associated with *T. asperellum* MSU007. Conversely, in the treatment involving the application of Terrachor Super-X chemical and H_2O, the hyphal growth originating from the sclerotia, which represents a filamentous form of *S. rolfsii*, exhibited a notably slower growth rate compared to the hyphae in the other experimental treatments. Subsequently, we investigated the longevity of efficacy of liquid formulations containing *T. asperellum* MSU007 in inhibiting *S. rolfsii* hyphal growth. These formulations consistently restricted hyphal expansion, even after extended storage, suggesting their potential for sustained control of *S. rolfsii* mycelium growth. Further research is needed to elucidate the mechanism of action and interactions between *T. asperellum* MSU007 and *S. rolfsii*. In contrast to previous studies focusing on solid-state fermentation, Rezende *et al.* [17] emphasized the advantages of submerged liquid fermentation for large-scale production of *Trichoderma* spp. They optimized key variables to enhance conidial production, with *T. asperelloides* LQC-96 demonstrating efficient parasitism of *S. sclerotiorum*, preventing sclerotium myceliogenic germination. This study provides essential insights into optimal fermentation conditions for maximizing conidial production of *T. asperelloides*, positioning it as a promising biofungicide against *S. sclerotiorum*. Finally, the study evaluated different *Trichoderma* formulations in inhibiting the germination o*f S. rolfsii* sclerotia. Formulation 4 and Terrachor Super-X exhibited the highest efficacy, with Formulation 4 displaying notable effectiveness even at lower concentrations. These findings suggest that Formulation 4 holds promise as a tool for controlling *S. rolfsii* sclerotia germination.

Table 3 The efficacy of the formulation to inhibit the germination of sclerotia.

Treatment	Rate	Sclerotia germination		
		Storage time		
		15 days	30 days	45 days
Formulation 1	4 mL/L	+	++	++
	5 mL/L	0	++	++
	6 mL/L	0	++	++
Formulation 2	4 mL/L	++	++	0
	5 mL/L	+	+	++
	6 mL/L	+	0	+
Formulation 3	4 mL/L	++	+	++
	5 mL/L	+	0	+
	6 mL/L	+	0	0
Formulation 4	4 mL/L	0	0	0
	5 mL/L	0	0	0
	6 mL/L	+	0	0
Terrrachor Super-X	3 mL/L	0	0	0
dH_2O	-	++	++	++

0 = no mycelial growth around the sclerotia
+ = mycelial growth around the sclerotia
++ = mycelial growth around the sclerotia and they grow in the formulation

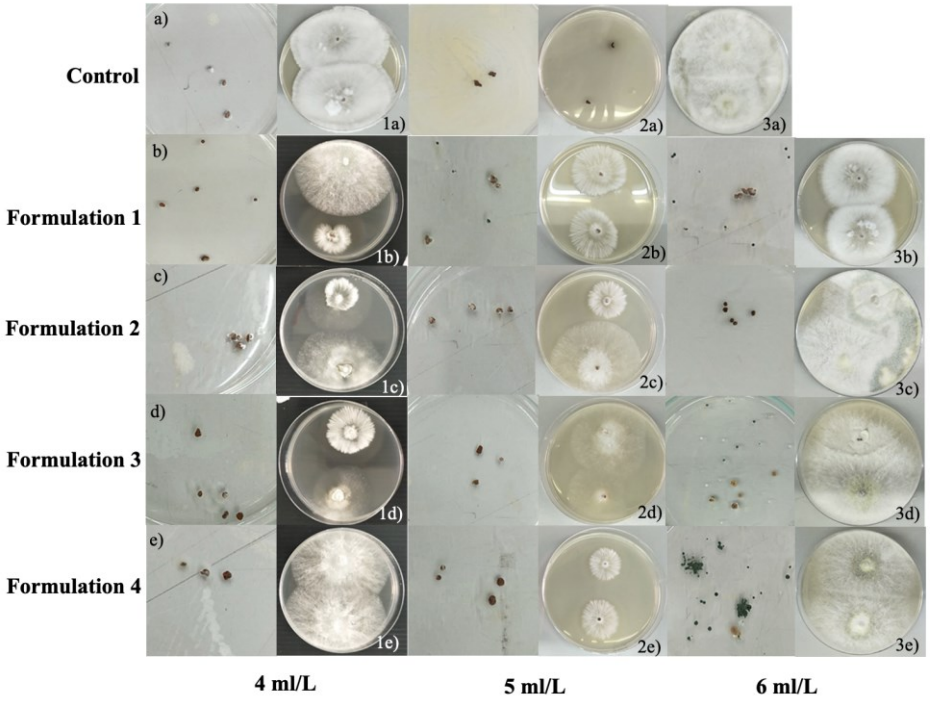

Figure 1 Efficacy of *T. asperellum* MSU007 liquid formulation in inhibiting sclerotia germination of *S. rolfsii* mycelium. On the left side of each plate, the inhibition of sclerotia germination in the formulation is shown. On the right side, the colony morphology is displayed after transfer to a new PDA plate. (a) Control: (1a) dH2O, (2a) Terrachor Super-X, (3a) *Trichoderma asperellum* MSU 007, (b) Formulation 1, (c) Formulation 2, (d) Formulation 3, (e) Formulation 4.

Conclusions

This study highlights the potential of *T. asperellum* MSU007 and its liquid formulations as effective biocontrol agents against *S. rolfsii*. The formulations demonstrated consistent viability and efficacy in inhibiting mycelium growth and sclerotia germination, with Formulation 4 and Terrrachor Super-X showing exceptional performance. The results emphasize the importance of formulation choice, with trehalose-based formulations exhibiting extended efficacy. Further mechanistic investigations are warranted to elucidate the precise mode of action and interactions between *T. asperellum* MSU007 and *S. rolfsii.* Overall, these findings offer promising insights into the development of sustainable and effective strategies for the management of *S. rolfsii*-induced crop diseases.

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References

[1] GE Harman, CR Howell, A Viterbo, I Chet and M Lorito. *Trichoderma* species-opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol*. 2004; **2**, 43-56.

[2] R Tyśkiewicz, A Nowak, E Ozimek and J Jaroszuk-Ściseł. *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. *Int. J. Mol. Sci.* 2022; **23**, 2329.

[3] L Selbmann, S Onofri, M Fenice, F Federici and M Petruccioli. Production and structural characterization of the exopolysaccharide of the Antarctic fungus *Phoma herbarum* CCFEE 5080. *Res in Microbiol.* 2002; **153**, 585-92.

[4] B Huang, H Jia, X Han, J Gou, C Huang, J Wang, J Wei, J Wang and C Zhang. Effects of biocontrol *Bacillus* and fermentation bacteria additions on the microbial community, functions and antibiotic resistance genes of prickly ash seed oil meal-biochar compost. *Bioresour. Tech.* 2021; **340**, 125668.

[5] M Khan, M Salman, SA Jan and ZK Shinwari. Biological control of fungal phytopathogens: A comprehensive review based on *Bacillus* species. *MOJ. Biol. Med.* 2021; **6**, 90-2.

[6] AK Mukherjee, AS Kumar, S Kranthi and PK Mukherjee. Biocontrol potential of three novel *Trichoderma* strains: isolation, evaluation and formulation. *3 Biotech* 2014; **4**, 275-81.

[7] A Sharma, PL Kashyap, AK Srivastava, YK Bansal and R Kaushik. Isolation and characterization of halotolerant bacilli from chickpea (*Cicer arietinum* L.) rhizosphere for plant growth promotion and biocontrol traits. *Eur. J. Plant Pathol*. 2019; **153**, 787-800.

[8] LV Kolombet, SK Zhigletsova, NI Kosareva, EV Bystrova, VV Derbyshev, SP Krasnova and D Schisler. Development of an extended shelf-life, liquid formulation of the biofungicide *Trichoderma asperellum*. *World J. Microbiol. Biotechnol*. 2008; **24**, 123-31.

[9] G Oros and Z Naár. Mycofungicide: *Trichoderma* based preparation for foliar applications. *American J. Plant Sci*. 2017; **8**, 113-25.

[10] G Naveenkumar and R Sendilkumar. Item analysis methodology to measure knowledge of farmer's on eco-friendly farm technology in rice cultivar. *J. Krishi Vigyan* 2015; **4**, 56-9.

[11] W Sutthisa, A Popranom, A Taddeetrakool and S Khankhum. Development of *Trichoderma* formulation and application to control durian anthracnose disease. *Trends Sci.* 2024; **21**, 7276.

[12] DAS Gebily, GAM Ghanem, MM Ragab, AA Ali, NEK Soliman and THA El-Moity. Characterization and potential antifungal activities of three *Streptomyces* spp. as biocontrol agents against *Sclerotinia sclerotiorum* (Lib.) de Bary infecting green bean. *Egypt. J. Biol. Pest Contr.* 2021; **31**, 33.

[13] W Sutthisa. Biological control properties of *Cyathus* spp. to control plant disease pathogens. *J. Pure Appl. Microbiol*. 2018; **12**, 1755-60.

[14] LG Silva, RC Camargo, GM Moura, PSO Nunes, C Dunlap and W Bettiol. Dual functionality of *Trichoderma*: biocontrol of *Sclerotinia sclerotiorum* and biostimulant of cotton plants. *Front. Plant Sci*. 2022; **13**, 983127.

[15] J Liu, SP Tian, BQ Li and QZ Qin. Enhancing viability of two biocontrol yeasts in liquid formulation by applying sugar protectant combined with antioxidant. *BioControl*. 2009; **54**, 817-24.

[16] J Yuan, Y Ruan, B Wang, J Zhang, R Waseem, Q Huang and Q Shen. Plant growth-promoting rhizobacteria strain *Bacillus amyloliquefaciens* NJN-6-enriched bio-organic fertilizer suppressed Fusarium wilt and promoted the growth of banana plants. *J. Agric. Food Chem.* 2013; **61**, 3774-3780.

[17] LC de Rezende, AL de Andrade Carvalho, LB Costa, B de Almeida Halfeld-Vieira, LG Silva, ZV Pinto, MAB Morandi, FHV de Medeiros, GM Mascarin and W Bettiol. Optimizing mass production of *Trichoderma asperelloides* by submerged liquid fermentation and its antagonism against *Sclerotinia sclerotiorum*. *World J. Microbiol. Biotechnol*. 2020; **36**, 113.

[18] MR Chowdhury, SF Ahmed, B Khalid, ZF Bony, JF Asha and MKA Bhuiyan. Biocontrol efficiency of microencapsulated *Trichoderma harzianum* coupled with organic additives against potato stem rot caused by *Sclerotium rolfsii*. *Plant Stress* 2023; **9**, 100181.