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Enhancing Stability and Efficacy of *Trichoderma* Bio-Control Agents Through Layer-by-Layer Encapsulation for Sustainable Plant Protection

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Agricultural fungicide pollution poses a significant environmental challenge and causes adverse effects on human health. Therefore, strategies to limit fungicide usage are of paramount importance. *Trichoderma* fungi, due to their antagonistic activity against various pathogenic fungi, have shown potential as a sustainable alternative to chemical fungicides. However, bio-control agents like *Trichoderma* are vulnerable to physical stimuli and show diminished efficacy during prolonged storage. To address these challenges, a mild and scalable encapsulation method for *Trichoderma* spores is introduced, employing a layer-by-layer (LbL) approach using biobased lignin derivatives. It is demonstrated that the LbL encapsulation technique significantly improved spore stability relative to naked spores, even under adverse conditions including extreme temperatures and prolonged exposure to Ultraviolet (UV) irradiation. Notably, encapsulated *Trichoderma* spores showed enhanced efficacy in cultivating tomato plants compared to naked spores. Additionally, the findings revealed that the *in planta* efficacy of encapsulated spores is dependent on the specific *Trichoderma* strain used. This study suggests that *Trichoderma* spores encapsulated with lignin through the LbL approach, are a promising and sustainable alternative to chemical fungicides with potential for commercialization.

1. Introduction

Fungal and bacterial infections in agriculture are a substantial worldwide economic burden and pose a threat to food security.[1,2] Plant pathogens result in a 30–40% loss in crop production annually, representing food that could have fed the one billion people lacking sufficient food supplies.[3,4] For instance, tomato plants are susceptible to over 200 diseases, with *Fusarium oxysporum* causing 10–80% yield loss per year.[5,6] Reducing food loss often relies on heavy use of chemical fungicides and pesticides, which are an inefficient and environmentally harmful strategy.[7,8] Preventive pesticide spraying of plants leads to pollution of the surroundings, damaging plants, and animals.[9] Moreover, pesticide pollution is a human health concern, as exposure increases the risk of several diseases, particularly for farmers who are in direct contact with these toxic compounds.[10] However, traceable amounts of pesticides are also found in food products and drinking water, which may affect the broader population.[8,9,11] Alternatives to spraying fungicides are therefore urgently needed to address these environmental and health related issues.

A broadly used strategy involves delivering fungicides to plants through the injection of fungicide-containing nanocarriers, for example by using chitosan,[12] hemicellulose,[13] cellulose,[14] or lignin as an encapsulation material.[15–18] It has been demonstrated that targeted delivery of fungicides reduces the required dose and the *in planta* transport of nanocarriers was previously studied.[19] Injecting plants with fungicide-containing nanocarriers limits fungicide pollution while offering effective protection from disease.[20,21] However, injecting fungicide nanocarriers into each plant is time-consuming, may affect the plant, and, if injected before blossoming, may leave toxic residues on the plant, which can later be transferred to animals or humans.[20]

Biological control agents (BCAs) are microorganisms that can control the growth of pathogens offering a sustainable, fungicide-free alternative.[22,23] *Trichoderma* fungi have been identified as particularly promising BCA.[24] *Trichoderma* improves crop yields by increasing access to nutrients, producing antibiotics, regulating plant hormones, and enhancing water acquisition rates.[25,26]

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Trichoderma has demonstrated increased shoot and root growth in tomato plants when compared to control plants.[25,27,28] Several other studies have demonstrated enhanced plant health and disease resistance after treatment with *Trichoderma* spores.[5,25,29,30] However, BCAs face significant drawbacks limiting broader application, due to their poor shelf-life and general instability.[31,32] These drawbacks can be addressed by developing encapsulation methods for *Trichoderma* spores.[33] For instance, their UV stability was improved by encapsulation with calcium alginate[34]; *Trichoderma* germination was delayed by using cellulose nanocrystals crosslinked with Ca^{2+} ions[35]; and shelf-life was improved by encapsulation into sodium alginate particles.[36] However, these encapsulation strategies create formulations in the millimeter range, which potentially can impact the *in planta* delivery and thereby limit the performance in agricultural applications. Furthermore, the strategies have only been evaluated in vitro, indicating that additional research is necessary to assess the potential of *Trichoderma* encapsulation.

As a sustainable polymer for encapsulation, lignin holds promise for various applications in agriculture. Lignin is an underutilized bioresource that can be obtained as a byproduct of paper production, making it ecofriendly and sustainable.[37,38] Lignin has shown promise as a sustainable biopolymer used in the treatment of Esca, a grapevine trunk disease. Single injections of fungicide-loaded lignin nanocarriers demonstrated fungicide efficacy for at least 4 years.[15,39] Furthermore, lignin was used to encapsulate *Trichoderma* spores and hydrophobic fungicides. As lignin degrades through secretion of ligninolytic enzymes, it acts as a protective layer without diminishing the ability of protecting against pathogens.[17,39,40] Lignin is particularly interesting for this type of encapsulation as it can self-assemble, allows controlled release, is widely available, and offers intrinsic antimicrobial, antioxidant, and UV-shielding properties.[37,38]

In this work, spores of *Trichoderma* strains were encapsulated with lignin to investigate the impact of encapsulation on spore stability. The encapsulation process involved applying alternate layers of cationic and anionic lignin polyelectrolytes. Both naked and encapsulated spores underwent exposure to high (50°C) and low (−20°C) temperatures, UVC and UVB light, as well as long-term storage. They were then analyzed by germination tests to assess whether encapsulation improved spore stability. Subsequently, both naked and encapsulated spores were utilized in a greenhouse study to treat tomato plants, aiming at determining if the encapsulated spores provided superior plant protection compared to naked spores. Overall, the encapsulated spores outperformed the naked spores, and it was evident that the strain type influenced their efficiency. In summary, the study reveals that lignin encapsulated *Trichoderma* spores are a promising sustainable alternative to traditional fungicides. This research enhances the potential of BCAs by extending their shelf-lives and confirming the *in planta* activity following soil application.

2. Results and Discussion

2.1. Synthesis and Characterization of Modified Biopolymers

Cationic and anionic polyelectrolytes were needed for the layer-by-layer (LbL) encapsulation of the *Trichoderma* spores.[39] To ensure biocompatibility and sustainability, lignin-based polymers were chosen as biopolymers, which are available as a waste material from paper production.[37,38,41] Cationic lignin was prepared from alkaline Kraft lignin by a substitution reaction with glycidyl trimethylammonium chloride (**Figure 1A**). This modification was performed using an improved literature protocol, which provided a high yield (above 85%) and opportunity for large scale production (more than 15 g in the university lab, which should not be regarded as a maximum).[42] After synthesis, cationic lignin was characterized by nuclear magnetic resonance spectroscopy (^{1}H NMR), Fourier transform infrared (FTIR), and gel permeation chromatography (GPC). The results can be found in Supporting Information (SI) Section 3. The ^{1}H NMR peak appearing at 3.2 ppm was assigned to the trimethylammonium group indicating a successful modification of lignin. Furthermore, the FTIR bands at 1263 and 1220 cm^{-1} indicated the formation of new ether linkages. The FTIR and ^{1}H NMR results align with literature demonstrating the successful isolation of the cationic lignin polymer.[39] Lignosulfonate (Figure 1B) is commercially available, however, it contains minor impurities from various carbohydrates and was therefore purified by dialysis before use. ^{1}H NMR was used to confirm the removal of the impurities (SI Section 3.3.2). The GPC analyses of the lignin polymers are shown in SI Section 3.4. Lignosulfonate had a M_w of 13 200 g mol^{-1}. However, it was not possible to measure the molecular weight of cationic lignin as the polymer eluted outside of the calibration curve, probably due to column interactions. Previous reports show an apparent M_w of 2400 g mol^{-1}.[39] The molar mass distributions were relatively broad as expected for lignin-based polymers.[43] The easy access and straightforward production of lignin polymers suitable for LbL encapsulation underline their potential in sustainable plant protection products.

2.2. Preparation and Characterization of LbL Encapsulated *Trichoderma* Spores

The encapsulation of microorganisms, spores and cells is challenging due to their poor stability when exposed to organic solvents, chemical compounds, and heat.[44] Therefore, it was necessary to choose a mild strategy for encapsulation of *Trichoderma* spores, which could remain anoxic to the spores. In a previous study, a mild layer-by-layer technique did not affect the viability of *Trichoderma* spores of a different strain.[39] Here, these findings are extended by investigating the stability of encapsulated spores under different conditions and their efficiency against infected tomato plants.

Prior to encapsulation, the toxicity of lignin polymers to the *Trichoderma* spores chosen for this study was evaluated. All three strains used in this study (called TRS14, TRS75, and TRS123, see Table S1 for details) were treated with cationic lignin or lignosulfonate and compared to an untreated sample before germination tests were performed. The results demonstrated no toxicity from the lignin polymers to the strains, making them suitable for layer-by-layer encapsulation (Figure S1, Supporting Information).

The layers were applied by suspending the spores in pure water before adding the polymer solution (0.2 wt.%). Since the spores' surfaces are negatively charged, the first polymer applied was the cationic lignin. Independent of the number of layers, the last layer was lignosulfonate to ensure an outer negative charge

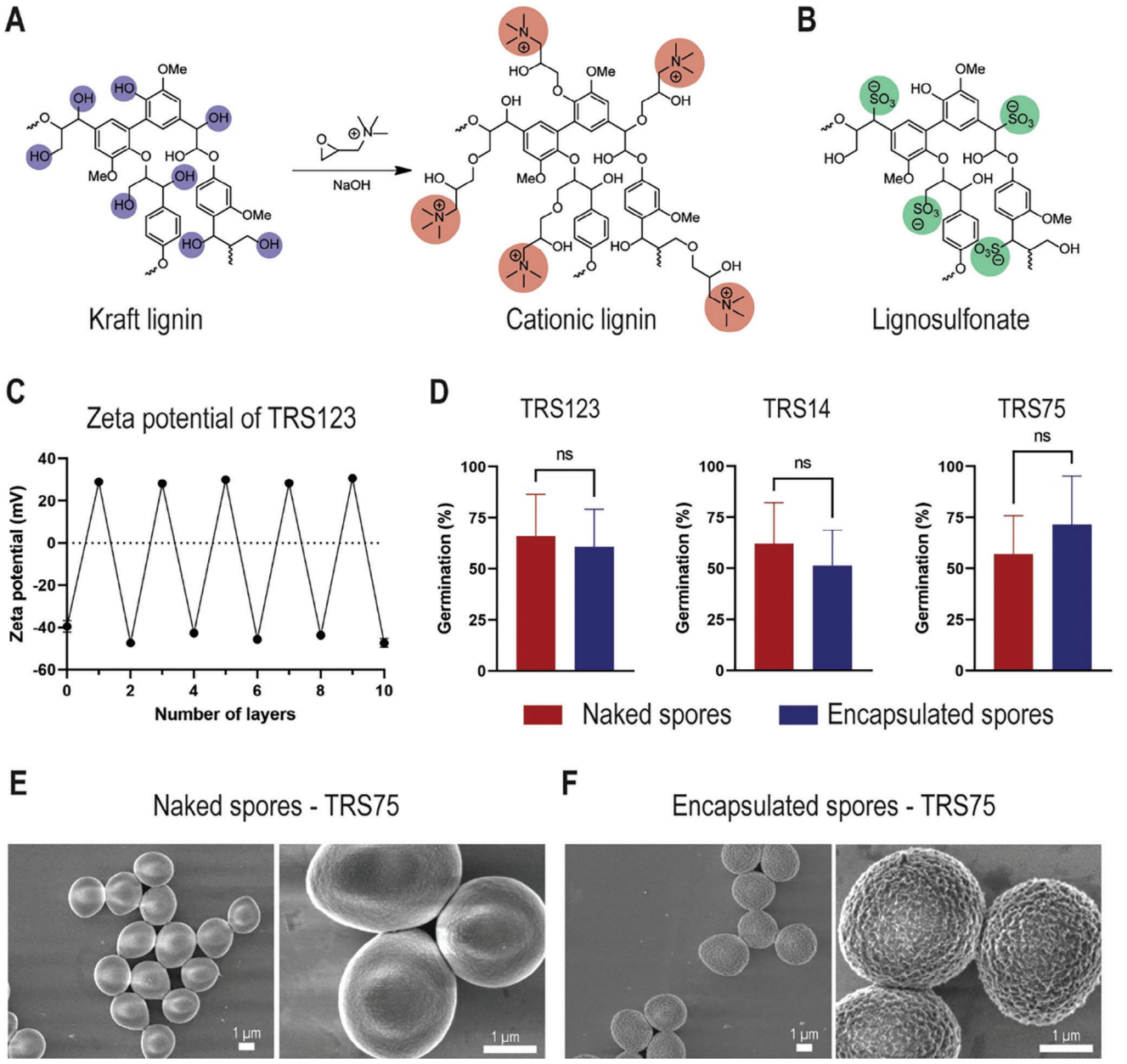

Figure 1. A) Schematic illustration of the synthesis of cationic lignin from Kraft lignin. Blue: Reactive hydroxyl groups. Red: Cationic functionality of lignin after modification. B) The structure of lignosulfonate with the anionic functionality marked in green. C) Zeta potential measurements after the absorption of each lignin layer of the TRS123 *Trichoderma* strain (see supporting information for the two other strains). The measurements were performed in triplicates. D) Germination of naked spores (red) and lignin encapsulated spores (blue) using 10 polymer layers. The results are shown for all three strains used: TRS123, TRS14, and TRS75. The experiment was performed in triplicates ($n = 3$) and repeated three times on different days ($N = 3$). P(TRS123) = 0.7567, P(TRS14) = 0.5170, P(TRS75) = 0.4547. P-values > 0.05 are non-significant (ns). The standard deviation includes the error of using Neubauer counting chamber (further elaborated on in SI Section 4.5) E) SEM of naked TRS75 with two different magnifications and F) SEM of encapsulated spores with 10 lignin polymer layers with two different magnifications.

like the naked spores. It was hypothesized that 10 layers would be sufficient to demonstrate a protective effect of the polymers. Therefore, the three *Trichoderma* strains were encapsulated with 10 layers of polymer. After each layer, the spores were analyzed by zeta potential measurements to ensure that the polymer was absorbed onto the spores. Figure 1C and Figure S3 (Supporting Information) show how the zeta potential varies between positive and negative values after addition of cationic or anionic polyelectrolyte layers, respectively. The effect of the 10 polymer layers on the spores' germination ability was analyzed by germination tests. The results of the germination tests for all three strains are shown in Figure 1D, demonstrating that germination did not significantly differ between the encapsulated and naked spores for any of the strains. To investigate this further, up to 50 layers were

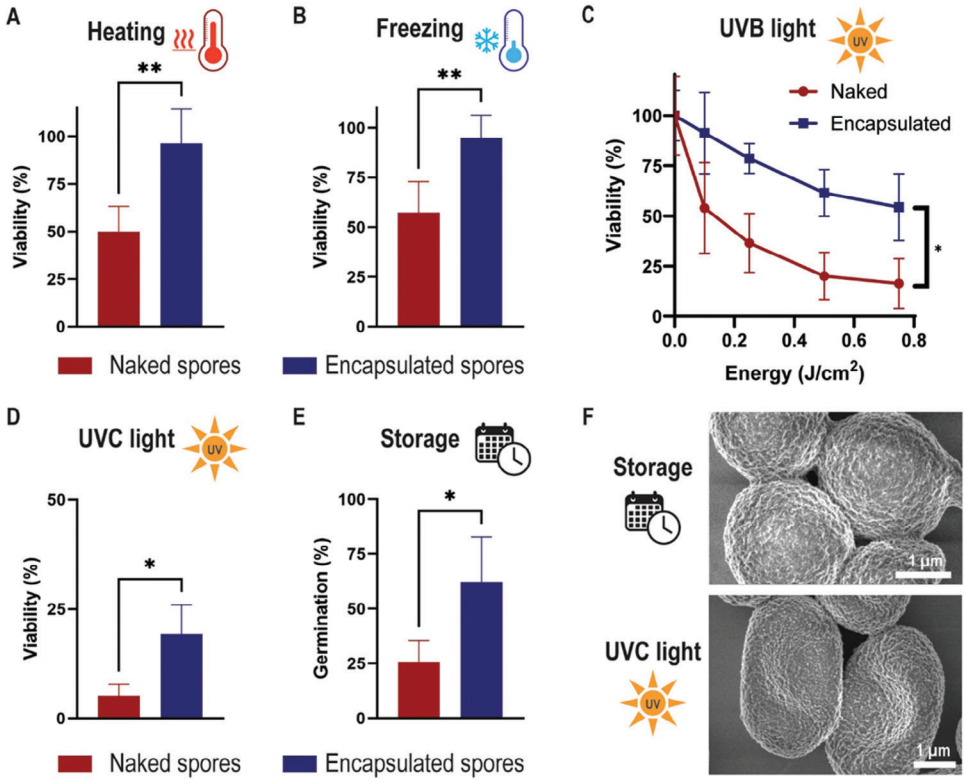

Figure 2. A–D) TRS123 *Trichoderma* spores naked (red) and encapsulated with 10 lignin polymer layers (blue) were treated with either A) heating at 50°C for 2 h, B) freezing at −20°C for 2 h, C) UVB light at different energies (in J cm^{-2}), or D) UVC light for 3 min. A–D) The treated samples were compared to non-treated samples that were normalized to 100% viability. Each experiment was performed in triplicates ($n = 3$) and repeated three times on different days ($N = 3$). The statistical significance was determined by a *t*-test (A,B,D) or a two-way ANOVA analysis (C) in GraphPad Prism. The *P*-values were as follows: A) $P = 0.0064$(**), B) $P = 0.0079$(**), C) see all *P*-values in Supporting Information Section 4.10.3, D) $P = 0.0269$(*). E) TRS14 *Trichoderma* spores naked (red) and encapsulated with 10 lignin polymer layers (blue) were stored at room temperature in 0.85% saline solution for 9 months followed by a germination test. The experiment was performed in triplicates ($n = 3$) and repeated three times on different days ($N = 3$). The statistical significance was determined by a *t*-test giving $P = 0.0182$(*). F) SEM of encapsulated TRS14 spores with 10 lignin layers after 9 months storage (top) and SEM of encapsulated TRS123 spores with 10 lignin layers after exposure to UVC light for 3 min (bottom).

applied, measuring the germination for every 10 layers (Figure S2, Supporting Information). Here, the germination was still not affected by the polymer layers using the herein applied strains. Combined, this supports that LbL using lignin-based polymers is a successful and mild strategy for encapsulation of *Trichoderma* spores with up to 50 layers. The encapsulated spores with 10 lignin layers were further characterized by scanning electron microscopy (SEM) and compared to naked spores. The original *Trichoderma* spores had a smooth surface (Figure 1E), however, upon addition of the polymer layers, the surface became rough indicating successful adsorption of the polymers (Figure 1F, the other strains are shown in Figure S4, Supporting Information).

2.3. Stability When Exposed to Physical Stimuli

The successful encapsulation of the *Trichoderma* spores using three different strains demonstrated that the mild and reproducible LbL method was effective with lignin-based polymers. It was hypothesized that encapsulating the spores would improve the stability making them more suitable as BCAs. One major drawback of using *Trichoderma* to treat plant diseases is the poor shelf-life and instability when exposed to various physical stimuli, such as heat, cold, and UV light.[18,19] For BCAs like *Trichoderma*, it is therefore important to improve stability when exposed to those stimuli. Experiments were conducted to investigate if the LbL-encapsulated spores would have improved stability to those stimuli.

2.3.1. Temperature Stability

The effect of extreme temperature variation on the viability of the spores was investigated. Here, the spores were exposed to either heating (50 °C) or freezing (−20 °C) for 2 hours and the viability of the naked and encapsulated spores were compared to untreated samples. The viability of the spores was measured by comparing the germination of untreated and treated spores, assuming that the untreated spores were 100% viable. The results are shown in **Figure** 2A,B. In both experiments encapsulated spores were significantly more stable than naked spores. While spores with 10 layers of polymer maintained almost full viability upon treatment, viability of the naked spores decreased to around 50% both when exposed to heat or freezing. The

improved stability to temperature variation is a huge advantage making spore storage less restrictive and thereby more accessible as a possible preventative treatment to control plant diseases.

2.3.2. UV Stability

The aromatic structure of lignin makes it efficient as protection against UV light, which has been demonstrated in coatings, cremes, and other lignin-containing polymer films.[45] A similar protective effect was anticipated for lignin-encapsulated *Trichoderma* against UV light. First, the spores were treated with UVB light at a wavelength of 302 nm at different energies (Figure 2C). Here, the viability of the spores decreased for both encapsulated and naked spores, however, the decrease was more severe for naked spores. Furthermore, the decrease in viability of the naked spores seemed to drop immediately (0.1 J cm^{-2}) to 50% while encapsulated spores were still almost fully viable at that timepoint. The decrease in viability of the naked spores was significant compared to encapsulated spores. A similar trend was observed when the spores were treated with UVC light at a wavelength of 235 nm for 3 min (Figure 2D). UVC light is normally used for sterilization, acting as a germicide.[46] Under UVC irradiation, viability of both samples significantly decreased. However, the encapsulated spores seemed to be more resistant to the UVC light and maintained more than 20% viability. The viability of naked spores was significantly lower and decreased to less than 5%. Furthermore, the lignin layers seemed to be intact after the UVC treatment as demonstrated by SEM analysis of the treated spores (Figure 2F (bottom)). Combined, these data suggest that lignin encapsulation protected the spores' viability during UV light exposure.

2.3.3. Shelf-Life Improvement

Trichoderma spores exhibit good shelf-lives for months if kept dry.[47] However, wet formulations of BCAs show a poor shelf-life and unwanted germination making them less suitable for farmers to store and use effectively.[47] Several strategies to increase BCAs shelf-life have been investigated but with only limited success.[47,48] The effect of lignin LbL-encapsulation strategy on storage time of *Trichoderma* spores was assessed. After 2 months, no differences were observed between encapsulated and naked spores when stored in saline solution at room temperature (Figure S5, Supporting Information). However, after 9 months of storage a significant decrease in germination was observed for naked spores while the encapsulated spores still maintained a similar degree of germination (Figure 2E). This is of high importance and especially the fact that the encapsulated spores were stable in saline solution is particularly interesting. To date formulations of *Trichoderma* spores have been prepared as freeze-dried powders or granules, which are inconvenient and damaging to the spores. Suspending the spores in saline solution is simpler and may suggest it is possible to avoid costly and damaging processing techniques. The *Trichoderma* spores were also analyzed with SEM after 9 months of storage (Figure 2F). This demonstrated that the lignin layers were intact, and it would therefore be expected to maintain protective properties against the various physical stimuli.

2.4. Plant Experiments

To evaluate the impact of lignin encapsulation on the *in planta* performance of *Trichoderma*, three successive greenhouse experiments were performed. Specifically, the greenhouse experiments were conducted with tomato plants, for which the growing medium was infested with *Fusarium oxysporum* f. sp. *lycopersici* (FOL), a common tomato plant pathogen. SI Section 4.12 provides a detailed description of the experiment.

The three *Trichoderma* strains each had different *in planta* performance, according to the greenhouse studies. The plant development was enhanced the most by treatment with *T. simmonsii* TRS75, especially the encapsulated spores (E-TRS75 **Figure 3**). In this case, the plants' weight and height were significantly higher than those of the control group (C Figure 3A,B). In addition, plants treated with encapsulated TRS75 spores showed a higher reduction in fusarium wilt symptoms than plants treated with naked TRS75 spores (N-TRS75 Figure 3C). In comparison to untreated plants, application of *T. atroviride* TRS14 to the growing medium resulted in a significant increase in plant height (Figure 3B) and a reduction in fusarium wilt symptoms (Figure 3C). However, unlike TRS75, encapsulation of TRS14 spores did not improve their efficacy. The lowest effect on plant growth was observed using spores of *T. gamsii* TRS123. This fungus positively affected tomato plant height compared to control plants, however, it did not impact the plant weight or fusarium wilt symptoms (Figure 3). Nonetheless, it was found that the encapsulated TRS123 spores significantly increased plant biomass (Figure 3A). Figure S6 (Supporting Information) contains photographs of the tomato plants treated with each of the *Trichoderma* strains in the greenhouse experiment.

The appropriate formulation is essential for a successful performance of BCAs in commercial agricultural conditions.[33,49,50] Our studies have demonstrated that the encapsulation of *Trichoderma* spores into lignin capsules ensured viability under different stress conditions while potentially improving *in planta* performance, as observed for TRS75. These findings were supported by the microbial analysis of the growing substrate. Here, it was found that encapsulated spores of TRS75 were the best colonizers, and significantly reduced *Fusarium* species prior to planting of tomato plants (**Table 1**). The microbiological analysis of the growing substrate added with fungi, followed by analysis of the plant rhizosphere, revealed that the number of *Trichoderma* propagules increased after application, compared to the initial added quantity of 10^4 cfu g^{-1} to the medium (**Table 2**). This was despite the substrate (not sterilized before use) already contained indigenous *Trichoderma* fungi. The encapsulation of the spores of TRS75 and TRS123 significantly increased their viability in the growing substrate and stimulated multiplication compared to other treatments. Two weeks after the application, the number of *Trichoderma* propagules in the substrates inoculated with TRS75 and TRS123 microcapsules increased 3 and 2.5-fold, respectively (compared to the control). For encapsulated TRS14 no significant effect on their viability in the substrate was observed compared to the naked spores. However, the number of *Trichoderma* in the substrate was higher for all encapsulated strains when compared to the control (Table 1). The improved effect of the lignin-encapsulated spores may be due to increased expression of cell wall-degrading enzymes, which are involved in biocontrol activi-

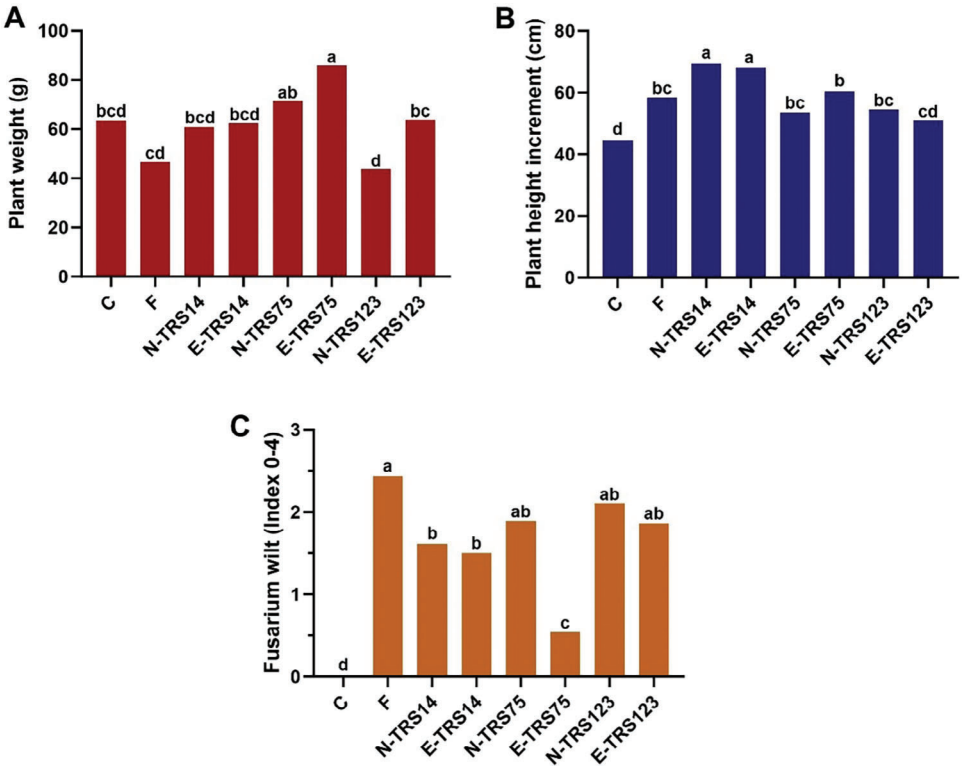

Figure 3. Growth parameters and fusarium wilt severity of tomato plants treated with encapsulated spores of Trichoderma strains. A) Plant weight, B) plant height increment, and C) fusarium wilt severity. C - control not treated; F - growing medium infested with Fusarium; N-TRS14, N-TRS75, N-TRS123—treatments with naked spores of *Trichoderma*; E-TRS14, E-TRS75, E-TRS123—treatments with encapsulated spores of *Trichoderma*. All growing media containing *Trichoderma* were infested with Fusarium. The figures represent the average values of three experiments. The same letters above the bars indicate no statistical difference according to Duncan test ($p = 0.05$).

Table 1. The number of *Trichoderma* spp. and *Fusarium* spp. in the growing media after two weeks of incubation, before planting of tomato plants. The values expressed as cfu g^{-1} of dry weight of the growing medium (g.m.).

Treatments	*Trichoderma* spp.	*Fusarium* spp.
	10^5 cfu g^{-1} g. m.	10^5 cfu g^{-1} g.m.
Control	4.63 ± 2.69 b	<1 d
Fusarium control	4.48 ± 2.52 b	4.70 ± 0.79 a
N-TRS14	7.07 ± 2.94 b	2.07 ± 0.27 bc
E-TRS14	6.55 ± 3.81 b	2.77 ± 0.46 b
N-TRS75	4.32 ± 1.37 b	1.22 ± 0.17 cd
E-TRS75	15.47 ± 8.45 a	1.54 ± 0.40 c
N-TRS123	3.82 ± 1.58 b	1.16 ± 0.29 cd
E-TRS123	11.68 ± 5.21 ab	0.98 ± 0.24 cd

The data represents average values of three experiments. Means followed by the same letter in columns are not significantly different according to Duncan test ($p = 0.05$).

Table 2. The number of *Trichoderma* spp. and *Fusarium* spp. in the rhizosphere of tomato plants growing in the media infested with *Fusarium oxysporum f.sp. Lycopersicon* and inoculated with Trichoderma encapsulated or naked spores. The values expressed as cfu g^{-1} of the roots.

Treatments	*Trichoderma* spp.	*Fusarium* spp.
	10^5 cfu g^{-1}	10^5 cfu g^{-1}
control	0.98 ± 0.26 b	<1 c
Fusarium control	0.71 ± 0.20 c	0.64 ± 0.12 b
N-TRS14	0.87 ± 0.22 bc	0.52 ± 0.09 b
E-TRS14	1.01 ± 0.26 b	0.54 ± 0.08 b
N-TRS75	1.13 ± 0.29 b	0.70 ± 0.09 b
E-TRS75	1.00 ± 0.27 b	0.33 ± 0.06 bc
N-TRS123	1.13 ± 0.32 b	0.57 ± 0.15 b
E-TRS123	1.39 ± 0.36 a	0.85 ± 0.30 a

The data represents average values of three experiments. Means followed by the same letter in columns are not significantly different according to Duncan test ($p = 0.05$).

ties by *Trichoderma* in the field, as reported earlier.[33,39] In ongoing studies of the antagonistic properties of the *Trichoderma* spp. strains TRS14, TRS75, and TRS123, the TRS75 strain appeared to exhibit a high enzymatic activity, especially for cellulase, chitinase and protease, while TRS14 produced mostly glucanase, and TRS123 for chitinase and protease.

The microbial analysis further indicated that all *Trichoderma* treatments significantly reduced the density of *Fusarium* spp. in the growing substrates infested with FOL. The strongest inhibitory effect was obtained with TRS75 and TRS123, which both decreased the number of *Fusarium* ca. 4-fold, while TRS14 resulted in a ca. 2-fold reduction. All encapsulated spores had no

significant influence on the effectiveness of *Fusarium* elimination in the substrate or the *Fusarium* incidence in tomato rhizosphere at the end of the experiment (Table 2). The rhizosphere was less colonized by *Fusarium* (10^4 cfu g^{-1} of roots) than the growing substrates (10^5 cfu g^{-1} of the substrate). Similarly, less *Trichoderma* colonies were isolated from tomato roots than from the substrate (Tables 1 and 2). More *Trichoderma* were isolated from the roots of plants grown in TRS-inoculated substrates than from the substrate infested with FOL alone, but these differences were not as significant. There were also no significant differences in roots colonization by encapsulated and naked spores, except for TRS123, where encapsulated spores were superior.

According to the review by Sharma and Sharma, the mechanism most involved in the interactions between *Trichoderma* and *Fusarium* in soil and rhizosphere is mycoparasitism, supported by production of antimicrobial volatiles and nonvolatile compounds.[51] Previous studies on the strains used here (Table S1, Supporting Information) showed that TRS14 indicated strong antagonism in vitro towards *Fusarium*, related to antibiotic compounds production, but also plant growth promotion and induction of systemic resistance. In the case of TRS75, high production of lytic enzymes may not only have a positive effect on the spore's germination, but also suggest mycoparasitism as a mechanism to suppress FOL in the growing substrate. TRS123 mostly produced volatiles, which might contribute to resistance induction in tomato plants, however, this effect was not significant. Nevertheless, these theories require additional research.

3. Conclusion

The encapsulation of *Trichoderma* spores with cationic and anionic lignin significantly improved the stability of the spores when exposed to high and low temperatures, retaining almost full viability. When exposed to UV light the viability was more affected, however, encapsulated spores were better protected against UVB and UVC light compared to naked spores. Furthermore, shelf-life was improved when left in saline solution for up to 9 months without compromising germination as observed for the naked spores. During the greenhouse experiments, encapsulated spores proved more effective than naked spores. However, the *in planta* performance was dependent on the strain of *Trichoderma*. Encapsulated spores of *T. simmonsii* TRS75 showed the most promise, by strongly reducing fusarium wilt symptoms and improving tomato plant growth in growing substrate infested with FOL. Spore encapsulation improved the performance of *T. gamsii* TRS123, however, to a lower extent than for TRS75, as a positive effect was only observed on the biomass of tomato plants. It was found that both TRS75 and TRS123, intensively colonized growing substrate when encapsulated spores were used. *T. atroviride* TRS14 indicated a positive influence on tomato plant growth, but encapsulation did not improve performance. Based on the *in planta* experiments, encapsulation using lignin can enhance efficacy and activity in biological control, a crucial attribute when dealing with an aggressive target pathogen. In conclusion, this study demonstrates the potential of lignin encapsulated *Trichoderma* spores as a sustainable alternative to chemical fungicides to treat plant infections and thereby improve crop yields.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

encapsulation, lignin, microcapsules, tomato plants, *Trichoderma*

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