

Article

Electrospun Cellulose Acetate Scaffolds with Electrospayed Chitooligosaccharides for *Bacillus subtilis* Delivery and Biocontrol

Nasko Nachev ¹, Petya Tsekova ¹, Donka Draganova ², Iliyana Valcheva ², Mariya Spasova ¹ and Olya Stoilova ^{1,*}

¹ Laboratory of Bioactive Polymers, Institute of Polymers, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria; nachev_n@polymer.bas.bg (N.N.); cekovapetya@polymer.bas.bg (P.T.); mspasova@polymer.bas.bg (M.S.)

² Biodinamika Ltd., 4000 Plovdiv, Bulgaria; donkadraganova@gmail.com (D.D.); valchevailiana1@gmail.com (I.V.)

* Correspondence: stoilova@polymer.bas.bg

Abstract

The increasing demand for sustainable agriculture necessitates the development of eco-friendly alternatives to chemical pesticides. This study reports the design and characterization of biodegradable fibrous mats for the delivery of *Bacillus subtilis*, a plant-beneficial biocontrol agent, using cellulose acetate (CA) scaffolds functionalized with chitooligosaccharides (COS). Electrospun CA fibers were coated by electrospaying with COS or COS/*B. subtilis* suspensions in a single-step process to produce open, porous bio-hybrid scaffolds. Scanning electron microscopy confirmed uniform fiber formation and successful deposition of COS and bacterial layers, while ATR-FTIR spectroscopy verified the chemical composition of the fibrous mats. Water contact angle measurements indicated a shift from hydrophobic to highly hydrophilic surfaces, enhancing microbial adhesion and moisture-mediated activation. Mechanical testing demonstrated that thin COS coatings slightly improved tensile strength without compromising flexibility. Viability assays confirmed that encapsulated *B. subtilis* remained viable and capable of sporulation, and dual-culture assays demonstrated effective inhibition of *Alternaria solani*, *Fusarium avenaceum*, and *Rhizoctonia solani*. These results indicate that the electrospun/electrospayed CA/COS platform provides a protective, sustainable, and effective delivery system for biocontrol agents. This approach offers a promising strategy for reducing reliance on synthetic pesticides while maintaining crop protection efficacy.

Keywords: cellulose acetate; electrospinning/electrospaying; chitooligosaccharides; *Bacillus subtilis*; biocontrol; biodegradable polymer scaffolds



Academic Editors: Carmen-Mihaela Popescu and Anamaria Irimia

Received: 19 January 2026

Revised: 11 February 2026

Accepted: 16 February 2026

Published: 18 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Global food security is increasingly challenged by plant pathogens, including fungi, bacteria, and viruses, which collectively cause substantial annual losses in crop yield and quality worldwide [1,2]. For decades, broad-spectrum synthetic pesticides have long been an essential component of conventional pest control. However, growing concerns regarding soil and water contamination, adverse effects on non-target beneficial organisms, and the rapid emergence of pesticide-resistant pathogen strains have rendered this approach progressively unsustainable [3,4]. These limitations highlight the urgent need for alternative plant protection strategies that ensure agricultural productivity while preserving environmental integrity and human health.

One of the key pillars of modern sustainable agriculture is the use of beneficial microorganisms within integrated pest management frameworks [5]. Microbial biocontrol

agents (BCAs) suppress plant diseases through natural antagonistic mechanisms, reducing dependence on chemical pesticides and mitigating associated ecological risks such as biodiversity loss and food-chain contamination [6,7]. Despite their promise, the widespread adoption of microbial BCAs remains constrained by limitations in formulation and delivery. In particular, poor environmental resilience—manifested as reduced survival under ultraviolet radiation, desiccation, temperature fluctuations, and mechanical stress, often leads to inconsistent field performance [8]. Additional challenges include inadequate retention on plant or soil surfaces, uncontrolled release profiles, limited shelf life, and scalability constraints, all of which hinder reliable and cost-effective agricultural application [9].

Among BCAs, the rhizobacterium *Bacillus subtilis*, a Gram-positive, spore-forming soil bacterium, has attracted considerable attention due to its broad-spectrum biocontrol activity and environmental robustness. Its efficacy derives from multiple complementary mechanisms, including competition for nutrients and ecological niches [10], the production of antimicrobial lipopeptides such as surfactin, iturin, and fengycin [11,12], and the induction of plant defense responses through induced systemic resistance [13]. Numerous studies have shown that lipopeptides produced by *B. subtilis* exhibit strong inhibitory activity against a broad range of fungal and bacterial phytopathogens [14]. In addition, its ability to form highly resilient spores further enhances its suitability for agricultural deployment, enabling survival under adverse environmental conditions while avoiding the persistence of harmful chemical residues [15]. To unlock the full potential of microbial BCAs such as *B. subtilis*, advanced delivery systems are required. These systems must protect microbial viability during storage and field application, enable controlled and localized release in the rhizosphere, and maintain a low environmental footprint [16]. Encapsulation within biodegradable polymeric matrices represents a promising strategy to address these challenges. Such matrices can provide a protective microenvironment that buffers external stressors, enhances microbial survival, and supports sustained metabolic activity and gradual release at targeted plant sites [17]. However, conventional formulations, such as liquid suspensions, wettable powders, or granules, often fail to provide sufficient environmental stability or controlled moisture interaction, resulting in a rapid loss of efficacy under field conditions.

In the design of such carriers, adherence to green and sustainable material principles is essential. Carrier materials should be biodegradable, biocompatible, derived from renewable resources, and should not contribute to secondary environmental pollution [18]. Polysaccharides, particularly chitoooligosaccharides (COSs), are highly attractive in this context, as they combine renewable origin with intrinsic bioactivity, including antimicrobial effects and the ability to elicit plant defense responses [19]. Chitosan and its oligosaccharide derivatives have been widely reported to induce plant defense-related enzyme activity and to enhance the performance of microbial formulations [20]. In parallel, cellulose-derived polymers such as cellulose acetate (CA) offer complementary advantages as carrier materials. CA retains the renewable cellulose backbone [21], while acetylation improves solubility [22] and processing versatility [23], making it well suited for advanced fabrication techniques such as electrospinning [24–26]. The literature highlights CA's tunable biodegradability (depending on the degree of substitution), favorable mechanical properties, and excellent film- and fiber-forming capability [27]. Compared with commonly used biodegradable polymers such as polylactic acid or alginate [28], CA offers a balanced combination of processability, environmental compatibility, and economic viability derived from abundant biomass resources.

Electrospun fibrous matrices are highly attractive as microbial delivery systems because their large surface area, interconnected porosity, and tunable surface chemistry facilitate microbial adhesion, moisture exchange, and controlled release. In our previous work,

we developed biohybrid systems based on electrospun poly(3-hydroxybutyrate) (PHB) fibers combined with chitosan films for microbial encapsulation, achieving high microbial viability and pronounced antifungal activity while fulfilling sustainability criteria [29]. On the basis of these findings, the present study advances toward a more renewable polymer matrix by employing cellulose acetate. Herein, we report the first successful fabrication of electrospun CA fibrous scaffolds combined with electrosprayed COS suspensions containing *Bacillus subtilis*, using a single-step electrospinning/electrospraying approach. The resulting biohybrid system comprises a porous microfibrillar CA network that provides mechanical integrity, high specific surface area, and interconnected porosity, while the COS coating enhances surface wettability, microbial compatibility, and plant defense-elicitor functionality. Within this architecture, CA serves as a biodegradable physical support for bacterial spores and vegetative cells, whereas COS supports microbial survival and facilitates moisture-mediated activation and release. Physicochemical and biological characterization demonstrated that the developed scaffolds fulfill key performance criteria, including structural stability under handling and application conditions, mechanical properties suitable for soil and foliar use, and surface morphology conducive to microbial attachment and viability. Encapsulated *B. subtilis* retained its ability to germinate, sporulate, and exert antagonistic activity against major phytopathogens of economic relevance. Collectively, these results demonstrate that the proposed fully bio-based CA/COS scaffolds represent a sustainable and effective alternative to conventional pesticide formulations. By combining renewable materials, gentle processing, and biological functionality, this approach contributes to reduced chemical inputs, protection of soil and water resources, and enhanced agroecosystem resilience. Further studies, including field-scale efficacy trials and biodegradation assessments under realistic agricultural conditions, will be required to support practical implementation.

2. Materials and Methods

2.1. Materials

Cellulose acetate (CA) with a molecular weight of 30,000 g/mol and a degree of substitution of 39.8% was purchased from Sigma-Aldrich (St. Louis, MO, USA). Chitoooligosaccharides (COSs), with a molecular weight range of 3000–5000 g/mol, were supplied by Kitto Life Co., Ltd. (Pyeongtaek-si, Gyeonggi-do, Republic of Korea). Analytical-grade acetone, used for electrospinning of CA, was obtained from Sigma-Aldrich (Darmstadt, Germany). All chemicals were used as received without further purification.

The biocontrol microorganism *Bacillus subtilis* was obtained from the culture collection of Biodinamika Ltd. (Plovdiv, Bulgaria). For spore production, the bacterium was cultured in Tryptic Soy Broth (TSB; Biolife, Milan, Italy) at 28 °C under constant agitation at 197 rpm for 120 h. Spores were collected by centrifugation at 6000 rpm for 15 min at 4 °C. Following two washes with sterile distilled water, the spore suspension was standardized to 1×10^{10} spores/mL.

The phytopathogenic fungal strains (*Alternaria solani*, *Fusarium avenaceum* and *Rhizoctonia solani*) were cultivated on Potato Dextrose Agar (PDA; Merck, Darmstadt, Germany) at 28 °C for seven days. The resulting cultures were then used to prepare standardized agar plugs, which served as inocula in antagonism assays to evaluate the inhibitory effects of the fibrous scaffolds.

2.2. Preparation of Electrospun/Electrosprayed Biohybrid Scaffolds

Biohybrid scaffolds were fabricated in a single step by combining the electrospinning of cellulose acetate (CA) with the simultaneous electrospraying of either a chitoooligosaccharide (COS) solution or a COS/*Bacillus subtilis* suspension. The resulting materials are denoted

as COS-on-CA and COS/*B. subtilis*-on-CA, respectively. A control mat of neat CA fibers was also produced by electrospinning without electrosprayed components.

A 10 wt% spinning solution of CA was prepared using an acetone/water mixture (80/20 *v/v*) as the solvent. This concentration and solvent composition were selected based on established protocols to ensure uniform, bead-free CA fiber formation [30,31]. For electrospraying, a 0.5 wt% aqueous COS solution was used. The bacterial suspension for the biohybrid material was prepared by thoroughly mixing 10 mL of the COS solution with 10 mL of the *B. subtilis* spore suspension to form a homogeneous COS/*B. subtilis* suspension.

Simultaneous electrospinning and electrospraying was performed using a setup with two syringe pumps (NE-300, New Era Pump Systems, Inc., Farmingdale, NY, USA) positioned at 180°. Both pumps were connected to a high-voltage power supply set at 25 kV. The CA solution was electrospun from a syringe fitted with a 19-gauge needle at a flow rate of 3 mL/h, while the COS solution or COS/*B. subtilis* suspension was electrosprayed from an identical syringe and needle setup at 1.5 mL/h. The tips of both needles were positioned 10 cm from a grounded rotating drum collector (diameter 45 mm, speed 1000 rpm). This configuration enabled the direct deposition of electrosprayed COS droplets and bacterial cells onto the forming CA fibers, producing a well-defined biohybrid mat. The entire process was carried out at ambient temperature (25 °C) and a relative humidity of 51%.

2.3. Characterization

The morphology of the fibrous samples and bacterial cells was examined using scanning electron microscopy (SEM). Prior to imaging with Jeol JSM-5510 (JEOL Co. Ltd., Tokyo, Japan), all samples were vacuum-coated with a thin gold layer for 60 s using Jeol JFC-1200 fine coater. Mean fiber diameter, standard deviation, and average bacterial cell length and width were determined by analyzing at least 30 fibers or cells from SEM micrographs using ImageJ software (version 1.54g).

The chemical composition of the fibrous scaffolds was analyzed by attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR). Spectra were recorded using an IRAffinity-1 spectrometer (Shimadzu Co., Kyoto, Japan) equipped with a diamond-crystal MIRacle™ ATR accessory (PIKE Technologies, Fitchburg, WI, USA). Measurements were collected over the spectral range of 600–4000 cm^{-1} at a resolution of 4 cm^{-1} . Post-processing was performed using IRsolution software (version 1.04) to correct for atmospheric water vapor and carbon dioxide contributions.

The surface wettability of the fibrous materials was evaluated by static water contact angle measurements at 25 °C using an Easy Drop DSA20E goniometer (Krüss GmbH, Hamburg, Germany). A 10 μL droplet of deionized water was deposited onto rectangular fibrous specimens (2 cm $\times$ 7 cm), excised parallel to the collector rotation direction. Contact angles were calculated using instrument-integrated image analysis software (v. 1.92.1.1.), and the reported values represent the average of ten independent measurements.

The mechanical properties of CA, COS-on-CA and COS/*B. subtilis*-on-CA mats were characterized by uniaxial tensile testing using an Instron 3344 universal testing machine operated with Bluehill Universal software (version 3.11) and equipped with a 50 N load cell. Tests were conducted at 21 °C with a crosshead speed of 10 mm/min. Specimens with approximate dimensions of 400 μm (thickness) $\times$ 60 mm (length) $\times$ 20 mm (width) were evaluated. Young's modulus (*E*, MPa), tensile strength (σ , MPa), and elongation at break (ϵ_B , %) were determined from the stress–strain curves, with at least ten specimens analyzed for each formulation.

2.4. Microbiological Evaluation and Morphometric Analysis

The viability of *Bacillus subtilis* encapsulated within electrospun CA-based fibrous mats functionalized with electrosprayed COS was evaluated using qualitative growth assays on solid Tryptic Soy Agar (TSA). Sterile biohybrid mats (16 mm diameter) were aseptically placed onto TSA plates and incubated at 28 °C. Bacterial growth, evidenced by colony formation, was observed within 72 h, confirming the preservation of *B. subtilis* viability. The ability of the encapsulated spores to germinate and regain metabolic activity was monitored over time. Bacterial growth, evidenced by the appearance of visible colonies and biofilm formation emerging directly from the fibrous matrix, was observed within 72 h. These observations confirm that the electrospinning and electrospraying processes did not compromise the biological integrity or viability of the spores. Control samples consisted of pristine electrospun CA mats and CA mats functionalized with COS only, which were subjected to identical microbiological evaluation.

The antagonistic activity of *Bacillus subtilis* against phytopathogenic fungi (*Alternaria solani*, *Fusarium avenaceum*, and *Rhizoctonia solani*) was evaluated using a dual-culture assay on solid Potato Dextrose Agar (PDA). A circular agar plug (7 mm in diameter) excised from an actively growing, 5-day-old fungal culture was placed on one side of a PDA plate. Biohybrid fibrous mats (16 mm in diameter) containing *B. subtilis* were positioned on the opposite side of the plate, at an equal distance from the plate edge. The Petri dishes were incubated at 28 °C for up to 7 days. Antifungal activity was assessed by monitoring the interaction between the bacterial and fungal colonies and by comparing the radial growth of the fungal mycelium in the presence of the biohybrid mats with that of the corresponding control plates. This experimental setup enabled evaluation of the diffusion of antifungal metabolites from the fibrous matrix toward the advancing fungal hyphae.

To ensure high precision in growth analysis, two perpendicular diameters of each fungal colony (d_1 and d_2) were measured. To account for potential asymmetrical mycelial expansion, the colony surface area (A , cm²) was calculated using the equation for the area of an ellipse (Equation (1)):

$$A = \pi \cdot \frac{d_1}{2} \cdot \frac{d_2}{2}, \quad (1)$$

where d_1 and d_2 represent the horizontal and vertical diameters (cm), respectively.

The Percentage of Inhibition of Radial Growth (PIRG) was determined to quantify the antifungal efficacy of the electrospun mats relative to the untreated control. PIRG was calculated based on the mean colony surface areas according to Equation (2):

$$\text{PIRG} = (A_c - A_t) / A_c \times 100, \quad (2)$$

where A_c is the mean surface area of the fungal colony in the control group and A_t is the mean surface area of the fungal colony in the treatment group.

2.5. Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (version 5; GraphPad Software, Inc., San Diego, CA, USA). Differences between groups were evaluated by one-way analysis of variance (ANOVA), followed by Bonferroni post hoc tests. All data are presented as the mean $\pm$ standard deviation (SD), and all calculations were conducted using the same software. Statistical significance was defined at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***). All antifungal assays were conducted in triplicate ($n = 3$) to ensure reproducibility. Colony diameters and calculated surface areas were used for quantitative analysis of fungal growth inhibition. The variability in these measurements was assessed through standard deviation values to evaluate the consistency and stability of the antimicrobial effect of the polymer–microbial system.

3. Results

The present study aimed to develop a new class of eco-friendly biohybrid materials for plant disease biocontrol using a one-step electrospinning/electrospraying strategy. Three types of materials were fabricated: (i) cellulose acetate (CA) mats produced by conventional electrospinning; (ii) COS-*on*-CA mats, in which CA fibers were surface-functionalized with COS; and (iii) COS/*B. subtilis*-*on*-CA mats, where CA fibers were functionalized with both COS and *B. subtilis* by simultaneous electrospinning and electrospraying. Based on our previous study employing poly(3-hydroxybutyrate) (PHB)-based systems functionalized with COS and *B. subtilis* [32], this study extends the approach toward cellulose-derived polymers. Cellulose acetate was selected as a renewable, biodegradable matrix derived from the most abundant natural polysaccharide, offering excellent processability and compatibility with electrospinning. The adopted fabrication strategy is simple, scalable, and cost-effective, enabling precise control over fiber morphology and surface functionalization while avoiding multistep post-processing routes commonly used in biohybrid material preparation.

3.1. Morphology of Fibrous Biohybrid Scaffolds

The surface morphology of the electrospun CA and the COS-functionalized CA mats produced by simultaneous electrospinning/electrospraying was observed using SEM (Figure 1). SEM images of the electrospun CA mats (Figure 1a) exhibited a randomly oriented, bead-free morphology forming a highly porous three-dimensional fibrous network, characteristic of well-optimized electrospinning conditions. A comparable fibrous architecture was retained after COS deposition (Figure 1b), indicating that the electrospraying process did not disrupt fiber formation. A thin COS coating was uniformly distributed along the CA fibers, with occasional delicate film bridges formed between neighboring fibers, preserving the overall porosity of the scaffolds and the open structure required for mass transfer and microbial activity (Figure 1b). Mats functionalized with COS and *Bacillus subtilis* (COS/*B. subtilis*-*on*-CA) also maintained the highly porous fiber network. Rod-shaped bacterial spores were clearly visible on the fiber surfaces and evenly distributed throughout the scaffold, confirming successful deposition and adhesion facilitated by the COS layer (Figure 1c). The inclusion of COS enhanced bacterial adhesion, providing a stable biohybrid layer while preserving the open fibrous morphology. The observed bacterial morphology and dimensions were consistent with those reported in earlier studies using COS/*B. subtilis* on PHB substrates [32], indicating that the change in polymer matrix did not adversely affect bacterial deposition or integrity. The presence of COS significantly enhanced bacterial adhesion to the CA fibers, demonstrating the effectiveness of the electrospraying-assisted functionalization approach in producing stable biohybrid scaffolds.

Quantitative analysis revealed that neat CA fibers had an average diameter of $0.849 \pm 0.213 \mu\text{m}$. Surface functionalization with COS resulted in a slight increase to $0.877 \pm 0.231 \mu\text{m}$, while the additional presence of COS/*B. subtilis* did not cause a further significant change in fiber diameter. This modest increase indicates the formation of a thin, conformal COS coating on the fiber surface without compromising fiber uniformity or morphology, thereby preserving the open fibrous network essential for mass transport, moisture penetration, and microbial activity.

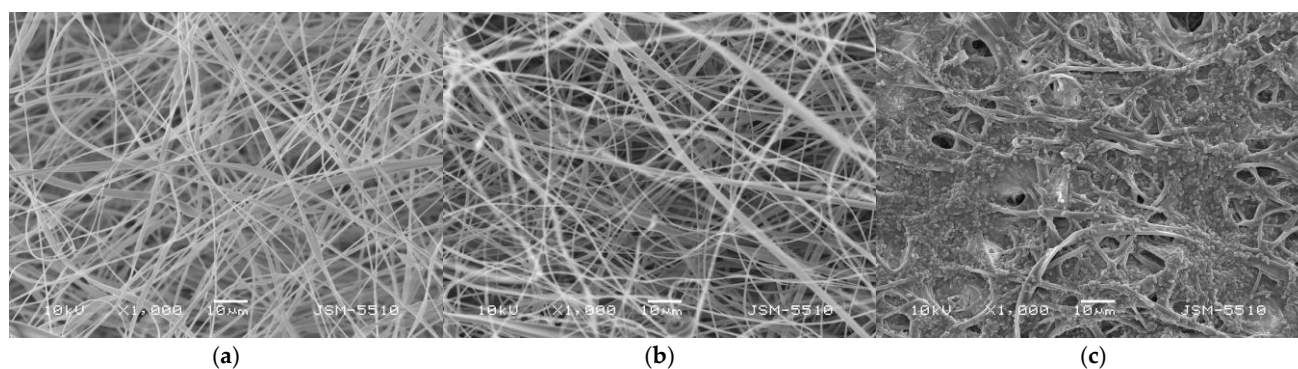


Figure 1. SEM micrographs of fibrous scaffolds: (a) CA mats; (b) COS-on-CA mats; (c) COS/*B. subtilis*-on-CA mats.

3.2. Surface Composition and Wettability

ATR-FTIR spectroscopy was used to analyze the chemical composition of the fabricated mats and to verify successful surface functionalization with COS (Figure 2). Electrospun CA fibers exhibited characteristic absorption bands at 1740 cm^{-1} , corresponding to C=O stretching vibrations of acetate groups, along with bands at 1368 and 1229 cm^{-1} attributed to $-\text{CH}_3$ and C–O stretching vibrations, respectively, and a band at 1036 cm^{-1} assigned to C–O–C stretching. The COS powder spectrum displayed characteristic absorption bands at 1647 cm^{-1} (amide I), 1568 cm^{-1} (amide II), and 1373 cm^{-1} (amide III), as well as bands at 1152 and 1063 cm^{-1} associated with C–O–C stretching vibrations of glucopyranose rings.

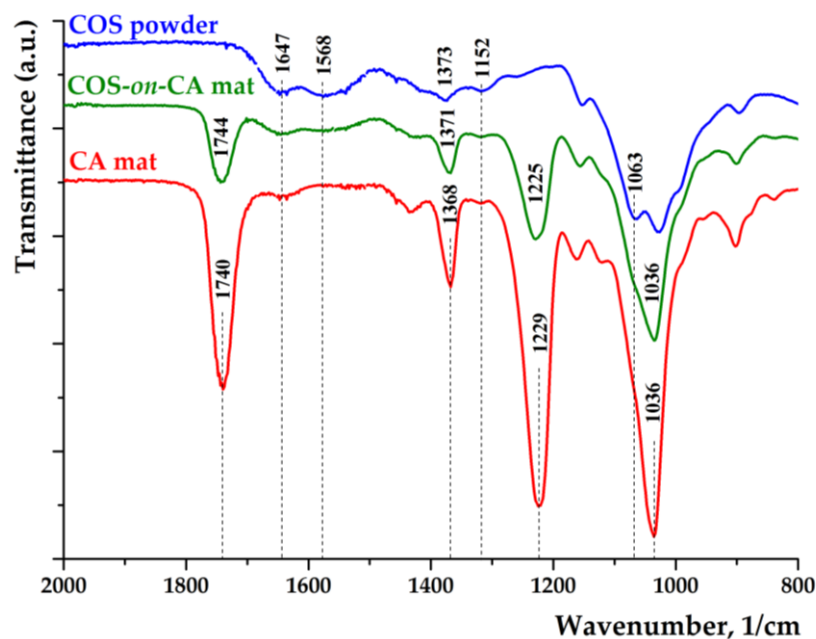


Figure 2. ATR-FTIR spectra of COS powder, electrospun CA mats, and COS-on-CA mats fabricated by combined electrospinning/electrospraying.

In the COS-on-CA mats, absorption bands characteristic of both CA and COS were clearly observed, confirming the successful deposition of COS onto the electrospun CA fibers (Figure 2). Notably, the CA-related carbonyl band shifted slightly from 1740 to 1744 cm^{-1} , while the bands at 1368 cm^{-1} (CA) and 1373 cm^{-1} (COS) merged and appeared at approximately 1371 cm^{-1} . Additionally, the band at 1229 cm^{-1} associated with CA shifted to 1225 cm^{-1} after COS functionalization. These minor shifts suggest weak intermolecular interactions, such as hydrogen bonding, between CA and COS at the

fiber surface rather than the formation of new covalent bonds. Importantly, the overall spectral features remained unchanged, indicating that the chemical integrity of the CA scaffold was preserved following electrospraying. These results confirm successful COS surface functionalization while maintaining the structural and chemical stability required for biohybrid microbial delivery platforms.

Surface wettability was evaluated to assess the suitability of the fabricated mats for biocontrol applications, as surface hydrophilicity plays a critical role in microbial activation, moisture uptake, and release behavior in aqueous environments. Water contact angle (WCA) measurements were therefore employed as a sensitive indicator of surface chemical modification following COS functionalization (Figure 3). Electrospun CA mats exhibited pronounced hydrophobic behavior, with static water contact angles exceeding 100° (Figure 3a). This behavior is consistent with the acetylated cellulose backbone and the rough fibrous morphology of electrospun CA. In contrast, CA mats functionalized with COS, either alone or in combination with *Bacillus subtilis*, showed immediate spreading of water droplets upon contact, resulting in contact angles of 0° (Figure 3b,c). This sharp transition from hydrophobic to highly hydrophilic surface behavior is attributed to the uniform deposition of water-soluble, low-molecular-weight COS on the CA fiber surfaces, in agreement with the FTIR results. The presence of COS introduces polar functional groups capable of strong interactions with water, thereby dramatically increasing surface wettability. Enhanced hydrophilicity is particularly advantageous for biocontrol applications, as it facilitates rapid scaffold hydration, supports microbial viability, and enables moisture-mediated activation and release of the encapsulated microorganisms under agricultural conditions, while also forming a localized “wet film” that improves contact between the biocontrol agent and the target pathogen.

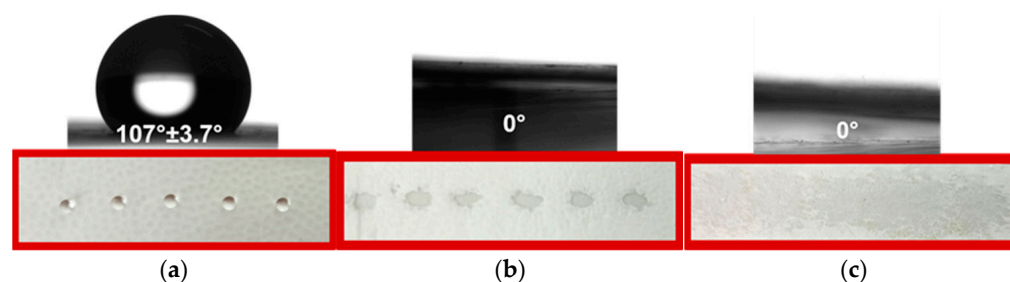


Figure 3. Water contact angle values and corresponding digital images of water droplets deposited on the surfaces of (a) CA mats, (b) COS-on-CA mats, and (c) COS/*B. subtilis*-on-CA mats.

3.3. Mechanical Properties

Mechanical integrity is an important requirement for fibrous materials intended for agricultural applications, as they may be subjected to handling, soil contact, humidity fluctuations, and mechanical stresses during use. The mechanical performance of CA, COS-on-CA, and COS/*B. subtilis*-on-CA mats was therefore evaluated by uniaxial tensile testing, and the corresponding stress–strain curves are presented in Figure 4. Electrospun CA mats exhibited a tensile strength of 1.14 ± 0.13 MPa, which is consistent with values previously reported for electrospun CA fibers (1–6 MPa) [33,34]. The relatively modest mechanical strength of the pristine CA mats can be attributed to the loosely packed fibrous architecture typical of electrospun materials, as well as to the semi-rigid backbone of cellulose acetate. Surface functionalization with COS resulted in a notable improvement in mechanical performance, with the tensile strength increasing to 1.45 ± 0.15 MPa. A further, although moderate, enhancement was observed for COS/*B. subtilis*-on-CA mats, which reached a tensile strength of 1.55 ± 0.14 MPa. This progressive increase is attributed to the formation of thin COS-based coatings that partially bridge adjacent fibers, thereby

improving stress transfer within the fibrous network. The bacterial cells themselves do not act as mechanical reinforcements; rather, their incorporation within the COS layer contributes indirectly to the formation of a more interconnected surface coating.

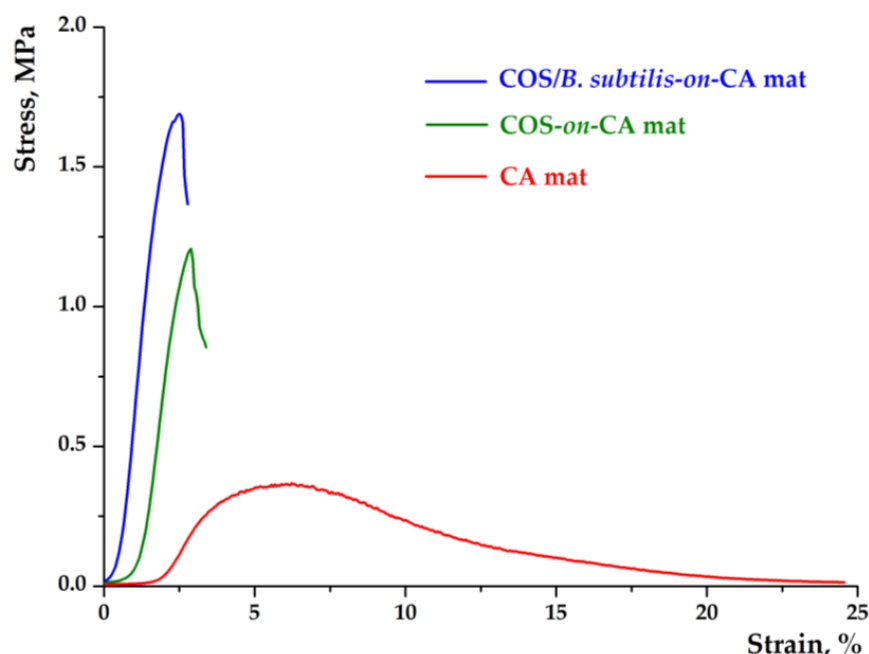


Figure 4. Stress–strain curves of CA, COS-on-CA, and COS/*B. subtilis*-on-CA fibrous mats.

Importantly, the observed improvements in tensile strength were achieved without inducing brittleness or compromising the intrinsic porosity and flexibility of the mats. These characteristics are essential for moisture transport, microbial viability, and effective interaction with plant surfaces, indicating that electrospray-assisted surface functionalization represents an effective strategy for enhancing the mechanical stability of electrospun biohybrid scaffolds. Moreover, the measured mechanical properties indicate that the mats are robust enough for practical handling, transport, and deployment under agricultural conditions, maintaining their structural integrity during bending, folding, and brief exposure to wind or humidity. The combination of improved tensile strength and preserved flexibility ensures that the mats can be applied effectively in the field while supporting biocontrol performance.

3.4. Bacterial Viability

Maintaining bacterial viability after immobilization is essential for the performance of biohybrid materials intended for biocontrol applications. The viability of *Bacillus subtilis* incorporated into COS-functionalized CA scaffolds was evaluated using agar plate assays (Figure 5). No microbial growth was observed for the CA or COS-on-CA control samples, confirming their sterility and the absence of bacterial contamination. In contrast, COS/*B. subtilis*-on-CA mats exhibited clear and reproducible colony formation after 72 h of incubation. Bacterial cells actively migrated from the fibrous scaffold onto the agar surface, where they displayed typical morphology and growth behavior characteristic of *B. subtilis*. Notably, sporulation was observed after 72 h, indicating that the encapsulated bacteria retained their physiological activity and capacity to complete their life cycle. These results demonstrate that the COS-functionalized CA mats provide a protective and supportive microenvironment that preserves bacterial viability during processing and enables subsequent growth and activation upon exposure to favorable conditions.

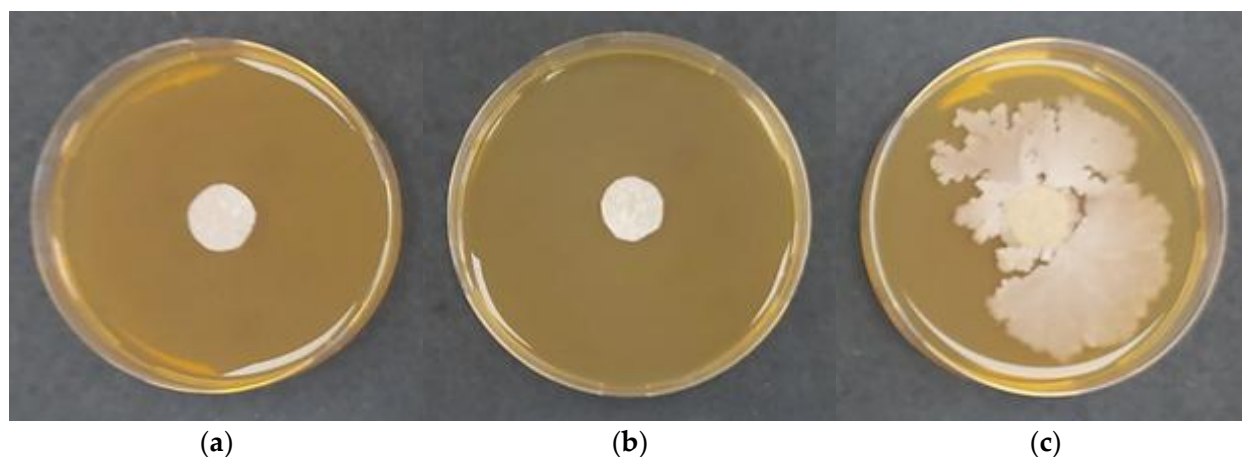


Figure 5. Digital images of the samples after 72 h of incubation: (a) CA mats; (b) COS-on-CA mats; (c) COS/*B. subtilis*-on-CA mats.

3.5. Antifungal Activity: Dual-Culture Assay

After confirming the viability of *B. subtilis* in the biohybrid mats, the antifungal potential of COS/*B. subtilis*-on-CA mats was evaluated against three economically relevant phytopathogens: *Alternaria solani*, *Fusarium avenaceum* and *Rhizoctonia solani*. These pathogens were selected to represent diverse survival strategies, ecological niches, and host ranges: *A. solani* is a foliar pathogen producing abundant airborne spores, *F. avenaceum* is a cosmopolitan pathogen capable of soil-borne and aerial infections and a major producer of mycotoxins, and *R. solani* is a soil-borne pathogen forming long-lived sclerotia. This triad allows for an assessment of the mats' broad-spectrum activity across different plant tissues and fungal survival forms, as well as their relevance to economically important crops and food safety. Dual-culture assays were performed using the agar block method, in which pre-grown fungal blocks were placed on one side of the Petri dish, while the fibrous mats in disk form were positioned at an equal distance opposite the fungal inoculum. Digital images of the results are shown in Figure 6a–l. As a negative control, pathogens were grown on agar without mats, representing unrestricted fungal growth.

For *Alternaria solani* (Figure 6a–d), the control showed unrestricted fungal expansion (Figure 6a). In contrast, CA and COS-on-CA mats showed no noticeable effect on the growth of the phytopathogenic fungi compared to the pathogen control (Figure 6b,c). In contrast, COS/*B. subtilis*-on-CA mats markedly suppressed fungal growth, as evidenced by reduced colony size and limited spread (Figure 6d). Bacterial cells migrated from the mats and effectively colonized the agar surface, maintaining their antagonistic activity. Similarly, for *Fusarium avenaceum* (Figure 6e–h), the control showed rapid radial growth (Figure 6e). CA and COS-on-CA mats did not cause any measurable reduction in fungal growth compared to the control (Figure 6f,g), whereas COS/*B. subtilis*-on-CA mats strongly inhibited fungal proliferation, demonstrating sustained antagonistic activity (Figure 6h). For *Rhizoctonia solani* (Figure 6i–l), extensive pathogen growth was observed in the control sample (Figure 6i), as well as in Petri dishes containing neat CA and COS-on-CA fibrous mats (Figure 6j,k), indicating that these materials did not noticeably affect fungal development. The COS/*B. subtilis*-on-CA mats effectively suppressed *R. solani* expansion, indicating robust biocontrol potential (Figure 6l).

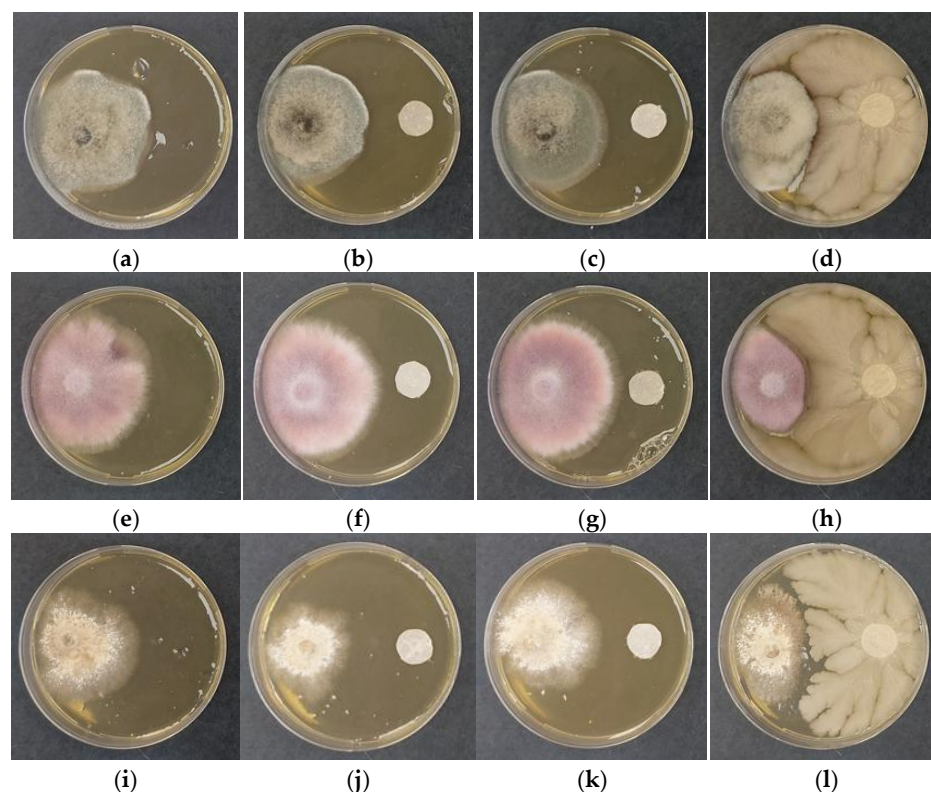


Figure 6. Digital images of dual-culture assays against three phytopathogens: (a–d) *Alternaria solani*, (e–h) *Fusarium avenaceum*, and (i–l) *Rhizoctonia solani*. For each pathogen: (a,e,i) control, (b,f,j) CA mats, (c,g,k) COS-on-CA mats, and (d,h,l) COS/*B. subtilis*-on-CA mats.

Morphometric characteristics and the percentage inhibition of radial growth (PIRG) of the tested phytopathogens in contact with the prepared scaffolds are presented in Table 1. The results show that the electrospun scaffolds loaded with beneficial bacteria consistently suppressed fungal growth across all tested pathogens. Incorporation of *Bacillus* spp. into the CA/COS mats significantly reduced colony surface area compared to both untreated controls and CA or COS-on-CA mats. As seen (Table 1), for *Alternaria solani*, the COS/*B. subtilis*-on-CA mats achieved a PIRG of 33.9%, limiting colony expansion to $15.1 \pm 1.1 \text{ cm}^2$. While *A. solani* displayed moderate resistance to the standalone CA and COS-on-CA mats, the presence of the microbial agent on the scaffold effectively inhibited radial growth. Obviously, the most pronounced inhibitory effect was observed against *Fusarium avenaceum*, where colony area was reduced by over 50% (Table 1) to $12.1 \pm 0.5 \text{ cm}^2$. The combination of high suppression and low variability underscores the stability and efficacy of the COS/*B. subtilis*-on-CA mats against this pathogen. In the case of *Rhizoctonia solani*, the presence of *Bacillus* spp. significantly restricted pathogen growth, resulting in a PIRG of 38.8%. The minimal standard deviation (Table 1) further highlights the reproducibility and reliability of the bioactive mats even against persistent soil-borne fungi. These results confirm that the CA/COS matrix functions primarily as a biocompatible carrier, while functionalization with *Bacillus* spp. establishes a potent, reliable antifungal barrier capable of restricting the development of diverse phytopathogens.

Overall, the results confirm that encapsulated *B. subtilis* retains its antifungal functionality across all tested phytopathogens. COS/*B. subtilis*-on-CA mats provide a biodegradable, sustainable, and highly effective delivery platform for microbial biocontrol agents in agricultural applications.

Table 1. Morphometric characteristics and percentage inhibition of radial growth (PIRG) of tested phytopathogens.

Phytopathogen	Scaffolds	Average Area *, cm ²	PIRG *, %
<i>Alternaria solani</i>	Control	22.8 ± 2.8	-
	CA mat	21.5 ± 1.1	5.4
	COS-on-CA mat	21.4 ± 0.8	6.2
	COS/ <i>B. subtilis</i> -on-CA mat	15.1 ± 1.1	33.9
<i>Fusarium avenaceum</i>	Control	28.7 ± 1.2	-
	CA mat	25.7 ± 1.0	10.5
	COS-on-CA mat	27.9 ± 1.5	2.6
	COS/ <i>B. subtilis</i> -on-CA mat	12.1 ± 0.5	57.7
<i>Rhizoctonia solani</i>	Control	20.3 ± 0.6	-
	CA mat	21.1 ± 1.2	n.a.
	COS-on-CA mat	22.9 ± 2.7	n.a.
	COS/ <i>B. subtilis</i> -on-CA mat	12.4 ± 0.2	38.8

* All antifungal assays were performed in triplicate ($n = 3$) to ensure reproducibility.

4. Discussion

Bacillus subtilis is widely recognized not only as a model microorganism but also for its environmentally friendly applications in agriculture, where it promotes plant growth, enhances resistance to phytopathogens, and contributes to soil health [35,36]. The present study demonstrates the successful development of a fully bio-based fibrous delivery platform for *B. subtilis* using electrospun cellulose acetate (CA) scaffolds functionalized with electrosprayed chitooligosaccharides (COSs). The selection of CA and COS was based on a combination of processability, biodegradability, and bioactivity. CA was chosen as the fibrous backbone due to its excellent electrospinnability from relatively low-toxicity, volatile organic solvents such as acetone, providing both mechanical integrity and a moderate, tunable degradation profile [37]. This profile differs from that of other common electrospinning polymers, such as PLA, which typically degrades more slowly and is more brittle [38], or PVA, which is highly hydrophilic and can dissolve rapidly in aqueous environments unless chemically crosslinked. COSs were selected for electrospraying due to their intrinsic antifungal and elicitor properties and their suitability for processing (e.g., readily water-soluble, low viscosity), unlike high-molecular-weight chitosan, which is more difficult to process [19].

The combination of electrospinning and electrospraying in a single-step process enabled the direct deposition of COS and bacterial cells onto forming CA fibers, producing thin, uniform, and defect-free mats while preserving the open, porous network essential for microbial viability and mass transport. COS plays a dual role in this architecture, acting as a natural adhesive that facilitates bacterial attachment to the fibers and creating a supportive microenvironment that preserves bacterial viability during storage, enhances resistance to environmental stress, and promotes activation upon hydration. This biodegradable CA/COS matrix thus provides a protective niche for *B. subtilis*, enabling gradual release and prolonged biological activity compared with free bacterial suspensions, which are prone to rapid degradation and wash-off. The gradual release refers to an architecture-driven mechanism rather than a quantified release rate. *B. subtilis* spores are mechanically entrapped within the three-dimensional CA fibrous network, enabling an initial activation of spores located at or near the fiber surface upon moisture exposure, followed by a progressive release of spores from the scaffold interior during subsequent wetting events. This spatial distribution helps prevent rapid wash-off and supports sustained biological activity after encapsulation.

Characterization confirmed the integrity and functionality of the scaffolds. The slight increase in fiber diameter and the presence of COS-specific ATR-FTIR bands indicated the formation of thin, conformal COS coatings without pore blockage, maintaining scaffold porosity for efficient moisture penetration and microbial release. Water contact angle measurements showed a strong hydrophilic transition after COS deposition, facilitating rapid hydration, bacterial activation, and sustained release in aqueous environments typical of soil and plant surfaces. Mechanical testing revealed that COS functionalization slightly enhanced tensile strength through the formation of thin inter-fiber films while preserving flexibility, an important property for agricultural handling and field deployment.

Viability assays confirmed that *B. subtilis* remained metabolically active after encapsulation, capable of germination and sporulation upon hydration. Dual-culture assays against *Alternaria solani*, *Fusarium avenaceum*, and *Rhizoctonia solani* demonstrated that COS/*B. subtilis*-on-CA mats maintained strong antagonistic activity, outperforming CA and COS-on-CA controls. These findings highlight that the developed biohybrid mats provide an effective and environmentally responsible alternative to conventional chemical pesticides while preserving microbial functionality. Quantitative analysis of antifungal activity, based on morphometric measurements and PIRG calculations, revealed a statistically significant reduction in colony surface area for the COS/*B. subtilis*-on-CA mats, with the strongest inhibitory effect observed against *Fusarium avenaceum*, reaching nearly 58% inhibition.

Overall, the integration of renewable polymeric scaffolds with COS and *B. subtilis* offers multiple synergistic advantages: enhanced microbial stability, hydrophilic surfaces for improved plant and soil interaction, mechanical robustness for practical use, and effective biocontrol activity against major plant pathogens. The single-step electrospinning/electrospraying approach represents a scalable, sustainable, and versatile platform for the fabrication of biohybrid delivery systems, bridging the gap between microbial formulation and practical agricultural deployment. COS/*B. subtilis*-on-CA mats therefore provide a promising route toward fully biodegradable, multifunctional biocontrol agents that reduce reliance on synthetic agrochemicals while maintaining crop protection efficacy.

5. Conclusions

A single-step electrospinning/electrospraying approach was successfully employed to fabricate cellulose acetate fibrous scaffolds functionalized with chitooligosaccharides and *Bacillus subtilis*. The resulting biohybrid mats combined structural integrity, hydrophilicity, and mechanical resilience, providing a supportive microenvironment for microbial viability and sustained release. Encapsulated *B. subtilis* maintained physiological activity and effectively inhibited key phytopathogens, including *Alternaria solani*, *Fusarium avenaceum*, and *Rhizoctonia solani*, under in vitro conditions. These results indicate that fully bio-based COS/*B. subtilis*-on-CA mats are a promising and environmentally responsible platform for biocontrol applications. Future studies will focus on greenhouse and field evaluations, controlled-release behavior, plant and soil interactions, and environmental fate to validate the long-term performance, safety, and practical applicability of these materials in sustainable agriculture.

Author Contributions: Conceptualization, M.S. and O.S.; methodology, M.S., O.S. and I.V.; formal analysis, N.N., P.T., D.D. and I.V.; investigation, N.N., P.T., D.D., I.V., M.S. and O.S.; data curation, N.N., I.V., M.S. and O.S.; writing—original draft preparation, N.N. and M.S.; writing—review and editing, O.S.; visualization, N.N., I.V., M.S. and O.S.; supervision, O.S.; project administration, O.S.; funding acquisition, O.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research and APC were funded by the European Union–NextGenerationEU, Investment C2.I2 “Increasing the innovation capacity of the Bulgarian Academy of Sciences in the field of green and digital technologies” under the Grant BG-RRP-2.011-0005-C01.

Data Availability Statement: Data are contained within the article.

Acknowledgments: Research equipment of Distributed Research Infrastructure INFRAMAT, part of the Bulgarian National Roadmap for Research Infrastructures, supported by the Bulgarian Ministry of Education and Science was partially used in this study.

Conflicts of Interest: Iliyana Valcheva and Donka Draganova were employed by the company Biodinamika Ltd. The remaining authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

References

1. Kalicharan, R.E.; Fernandez, J. Triple Threat: How Global Fungal Rice and Wheat Pathogens Utilize Comparable Pathogenicity Mechanisms to Drive Host Colonization. *Mol. Plant-Microbe Interact.* **2025**, *38*, 173–186. [CrossRef]
2. Ristaino, J.B.; Anderson, P.K.; Bebber, D.P.; Brauman, K.A.; Cunniffe, N.J.; Fedoroff, N.V.; Finegold, C.; Garrett, K.A.; Gilligan, C.A.; Jones, C.M.; et al. The Persistent Threat of Emerging Plant Disease Pandemics to Global Food Security. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2022239118. [CrossRef] [PubMed]
3. Ankit; Saha, L.; Kishor, V.; Baudh, K. Impacts of Synthetic Pesticides on Soil Health and Non-targeted Flora and Fauna. In *Ecological and Practical Applications for Sustainable Agriculture*; Baudh, K., Kumar, S., Singh, R., Korstad, J., Eds.; Springer: Singapore, 2020; pp. 1–27. [CrossRef]
4. Sud, D.; Kumar, J.; Kaur, P.; Bansal, P. Toxicity, Natural and Induced Degradation of Chlorpyrifos. *J. Chil. Chem. Soc.* **2020**, *65*, 4807–4816. [CrossRef]
5. Ilieva, T.; Karova, A.; Ivanova, M. Sustainable Agriculture through Integrated Pest Management: Strategies for Effective Implementation. In *Climate Crisis and Resilient Transportation Systems*; Nathanail, E.G., Gavanis, N., Adamos, E., Eds.; Springer: Cham, Switzerland, 2025; pp. 511–520. [CrossRef]
6. Villavicencio-Vásquez, M.; Espinoza-Lozano, F.; Espinoza-Lozano, L.; Coronel-León, J. Biological Control Agents: Mechanisms of Action, Selection, Formulation and Challenges in Agriculture. *Front. Agron.* **2025**, *7*, 1578915. [CrossRef]
7. Zhou, W.; Arcot, Y.; Medina, R.F.; Bernal, J.; Cisneros-Zevallos, L.; Akbulut, M.E.S. Integrated Pest Management: An Update on the Sustainability Approach to Crop Protection. *ACS Omega* **2024**, *9*, 41130–41147. [CrossRef]
8. Shaili, S.J.; Kabiraj, U.K.; Mahedi, M. Fungal Biocontrol in Agriculture: A Sustainable Alternative to Chemical Pesticides—A Comprehensive Review. *World J. Adv. Res. Rev.* **2025**, *26*, 2305–2316. [CrossRef]
9. Bonaterra, A.; Badosa, E.; Cabrefiga, J.; Francés, J.; Montesinos, E. Prospects and Limitations of Microbial Pesticides for Control of Bacterial and Fungal Pomefruit Tree Diseases. *Trees* **2011**, *26*, 215–226. [CrossRef]
10. Li, X.; Chen, Y.; Yang, S.; Zhou, Y.; Yang, C. Whole Genome-Sequence Analysis of *Bacillus subtilis* Strain KC14-1 with Broad-Spectrum Antifungal Activity. *BMC Genom.* **2025**, *26*, 319. [CrossRef]
11. Fan, H.; Zhang, Z.; Li, Y.; Zhang, X.; Duan, Y.; Wang, Q. Biocontrol of Bacterial Fruit Blotch by *Bacillus subtilis* 9407 via Surfactin-Mediated Antibacterial Activity and Colonization. *Front. Microbiol.* **2017**, *8*, 1973. [CrossRef]
12. Ongena, M.; Jacques, P.; Touré, Y.; Destain, J.; Jabrane, A.; Thonart, P. Involvement of Fengycin-Type Lipopeptides in the Multifaceted Biocontrol Potential of *Bacillus subtilis*. *Appl. Microbiol. Biotechnol.* **2005**, *69*, 29–38. [CrossRef]
13. Wang, X.Q.; Zhao, D.L.; Shen, L.L.; Jing, C.L.; Zhang, C.S. Application and Mechanisms of *Bacillus subtilis* in Biological Control of Plant Disease. In *Role of Rhizospheric Microbes in Soil*; Meena, V., Ed.; Springer: Singapore, 2018; pp. 247–266. [CrossRef]
14. Hazarika, D.J.; Goswami, G.; Gautam, T.; Parveen, A.; Das, P.; Barooah, M.; Boro, R.C. Lipopeptide Mediated Biocontrol Activity of Endophytic *Bacillus subtilis* against Fungal Phytopathogens. *BMC Microbiol.* **2019**, *19*, 71. [CrossRef]
15. Hashem, A.; Tabassum, B.; Abd_Allah, E.F. *Bacillus subtilis*: A Plant-Growth Promoting Rhizobacterium That Also Impacts Biotic Stress. *Saudi J. Biol. Sci.* **2019**, *26*, 1291–1297. [CrossRef]
16. Stefan, M.; Munteanu, N.; Dunca, S. Plant-Microbial Interactions in the Rhizosphere-Strategies for Plant Growth—Promotion. *J. Exp. Mol. Biol.* **2012**, *13*, 87. Available online: <https://api.semanticscholar.org/CorpusID:86117801> (accessed on 11 November 2025).
17. John, R.P.; Tyagi, R.D.; Brar, S.K.; Surampalli, R.Y.; Prévost, D. Bio-Encapsulation of Microbial Cells for Targeted Agricultural Delivery. *Crit. Rev. Biotechnol.* **2010**, *31*, 211–226. [CrossRef] [PubMed]
18. Malusa, E.; Pinzari, F.; Canfora, L. Efficacy of Biofertilizers: Challenges to Improve Crop Production. In *Microbial Inoculants in Sustainable Agricultural Productivity*; Singh, D.P., Singh, H.B., Prabha, R., Eds.; Springer: New Delhi, India, 2016; pp. 17–40. [CrossRef]

19. Mawale, K.S.; Giridhar, P.; Johnson, T.S. Chitosan: A Versatile Polymer for Enhancing Plant Bioactive Accumulation, Managing Plant Diseases, and Advancing Food Preservation Technologies. *Int. J. Biol. Macromol.* **2025**, *308*, 142081. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Lee, P.; Lin, X.; Khan, F.; Bennett, A.E.; Winter, J.O. Translating Controlled Release Systems from Biomedicine to Agriculture. *Front. Biomater. Sci.* **2022**, *1*, 1011877. [\[CrossRef\]](#)
21. Teacă, C.-A.; Stanciu, C.-M.; Tanasă, F.; Nechifor, M. Ionic Liquids for Enhanced Enzymatic Saccharification of Cellulose-Based Materials. In *Nanotechnology in the Life Sciences*; Abd-Elsalam, K.A., Ed.; Springer: Cham, Switzerland, 2020; pp. 113–135. [\[CrossRef\]](#)
22. Ma'ruf, A.; Purnawanto, A.M.; Rimatunnisa, R. Synthesis of Cellulose Acetate from Rice Husk Cellulose by Ultrasound-Assisted Acetylation. *BioResources* **2023**, *18*, 4688–4698. [\[CrossRef\]](#)
23. Bouftou, A.; Aghmih, K.; Majid, S. Elaboration and Characterization of Cellulose Acetate Film with Natural Plasticizer. In *Recent Advances in Environmental Science from the Euro-Mediterranean and Surrounding Regions*, 4th ed.; Advances in Science, Technology & Innovation; Ksibi, M., Sousa, A., Hentati, O., Chenchouni, H., Velho, J.L., Negm, A., Rodrigo-Comino, J., Hadji, R., Chakraborty, S., Ghorbal, A., Eds.; Springer: Cham, Switzerland, 2022; pp. 639–642. [\[CrossRef\]](#)
24. Tsiptsias, C.; Sakellariou, K.G.; Tsvintzelis, I.; Papadopoulou, L.; Panayiotou, C. Preparation and Characterization of Cellulose Acetate-Fe₂O₃ Composite Nanofibrous Materials. *Carbohydr. Polym.* **2010**, *81*, 925–930. [\[CrossRef\]](#)
25. Kalwar, K.; Shen, M. Electrospun Cellulose Acetate Nanofibers and Au@AgNPs for Antimicrobial Activity—A Mini Review. *Nanotechnol. Rev.* **2019**, *8*, 246–257. [\[CrossRef\]](#)
26. Wu, S.; Qin, X.; Li, M. The Structure and Properties of Cellulose Acetate Materials: A Comparative Study on Electrospun Membranes and Casted Films. *J. Ind. Text.* **2013**, *44*, 85–98. [\[CrossRef\]](#)
27. Aziz, T.; Farid, A.; Haq, F.; Kiran, M.; Ullah, A.; Zhang, K.; Li, C.; Ghazanfar, S.; Sun, H.; Ullah, R.; et al. A Review on the Modification of Cellulose and Its Applications. *Polymers* **2022**, *14*, 3206. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Topuz, F.; Uyar, T. Electrospinning of Sustainable Polymers from Biomass for Active Food Packaging. *Sustain. Food Technol.* **2024**, *2*, 1266–1296. [\[CrossRef\]](#)
29. Krastev, V.; Stoyanova, N.; Valcheva, I.; Draganova, D.; Naydenov, M.; Spasova, M.; Stoilova, O. Development of Chitosan-Coated Electrospun Poly(3-hydroxybutyrate) Biohybrid Materials for Growth and Long-Term Storage of *Bacillus subtilis*. *Polysaccharides* **2024**, *5*, 698–714. [\[CrossRef\]](#)
30. Tsekova, P.; Stoilova, O. Fabrication of Electrospun Cellulose Acetate/Nanoclay Composites for Pollutant Removal. *Polymers* **2022**, *14*, 5070. [\[CrossRef\]](#)
31. Spasova, M.; Manolova, N.; Rashkov, I.; Tsekova, P.; Georgieva, A.; Toshkova, R.; Markova, N. Cellulose Acetate-Based Electrospun Materials with a Variety of Biological Potentials: Antibacterial, Antifungal and Anticancer. *Polymers* **2021**, *13*, 1631. [\[CrossRef\]](#)
32. Stoyanova, N.; Nachev, N.; Naydenov, M.; Valcheva, I.; Spasova, M.; Stoilova, O. Biocontrol Potential of Poly(3-hydroxybutyrate) Fibers Functionalized with Chitooligosaccharide/*Bacillus subtilis* Using Electrospinning and Electrospraying. *Polymers* **2025**, *17*, 692. [\[CrossRef\]](#)
33. Chen, H.-L.; Lin, L.-Y.; Pham, U.; Lee, C.-K.; Hsiao, W. Development of Electrospun Nanofibrous Composite Membrane of Tannic Acid–Cellulose Acetate: Advancing Antimicrobial and Free Radical Scavenging Activity. *Carbohydr. Polym. Technol. Appl.* **2025**, *9*, 100666. [\[CrossRef\]](#)
34. Almeida, D.; Duarte, E.; Hashimoto, E.; Turbiani, F.; Muniz, E.; Souza, P.; Gimenes, M.; Martins, L. Development and Characterization of Electrospun Cellulose Acetate Nanofibers Modified by Cationic Surfactant. *Polym. Test.* **2020**, *81*, 106206. [\[CrossRef\]](#)
35. Blake, C.; Christensen, M.N.; Kovács, Á.T. Molecular Aspects of Plant Growth Promotion and Protection by *Bacillus subtilis*. *Mol. Plant-Microbe Interact.* **2020**, *34*, 15–25. [\[CrossRef\]](#)
36. Bi, Y.; Zhou, B.; Ren, P.; Chen, X.; Zhou, D.; Yao, S.; Fan, D.; Chen, X. Effects of *Bacillus subtilis* on Cotton Physiology and Growth under Water and Salt Stress. *Agric. Water Manag.* **2024**, *303*, 109038. [\[CrossRef\]](#)
37. Puls, J.; Wilson, S.A.; Höltzer, D. Degradation of Cellulose Acetate-Based Materials: A Review. *J. Environ. Polym. Degrad.* **2010**, *19*, 152–165. [\[CrossRef\]](#)
38. Auras, R.; Harte, B.; Selke, S. An Overview of Polylactides as Packaging Materials. *Macromol. Biosci.* **2004**, *4*, 835–864. [\[CrossRef\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.