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Bark Extract of *Uncaria tomentosa* L. for the Control of Strawberry Phytopathogens †

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Abstract: Gray mold (*Botrytis cinerea* Pers.), crown and fruit rot (*Phytophthora cactorum* (Lebert and Cohn) J.Schröt), and verticillium wilt (*Verticillium dahliae* Kleb.) are among the main diseases that affect the strawberry crop. In the study presented herein, the bark extract of *Uncaria tomentosa* (Willd. ex Schult.) DC, popularly known as "cat's claw", has been evaluated for its capability to act as a sustainable control method. The bioactive compounds present in the aqueous ammonia extract were characterized by gas chromatography–mass spectroscopy, and the antimicrobial activity of the extract—alone and in combination with chitosan oligomers (COS)—was assessed in vitro and as a coating for postharvest treatment during storage. Octyl isobutyrate (30.7%), 19α methyl-2-oxoformosanan-16-carboxylate (9.3%), tetrahydro-2-methyl-thiophene (4.7%), and α-methyl manofuranoside (4.4%) were identified as the main phytoconstituents. The results of in vitro growth inhibition tests showed that, upon conjugation of the bark extract with COS, complete inhibition was reached at concentrations in the 39–93.75 $\mu g \cdot mL^{-1}$ range, depending on the pathogen. Concerning the effect of the treatment as a coating to prolong the storage life and control decay during post-harvest storage, high protection was observed at a concentration of 1000 $\mu g \cdot mL^{-1}$. Because of this effectiveness, higher than that attained with conventional synthetic fungicides, the bark extracts of cat's claw may hold promise for strawberry crop protection.

Keywords: cat's claw; chitosan oligomers; coating; crown rot; GC–MS; gray mold; leather rot; postharvest; verticillium wilt

1. Introduction

Strawberry (*Fragaria* × *ananassa* (Duchesne ex Weston) Duchesne ex Rozier) is a widely consumed berry throughout the world. However, it is susceptible to certain fungal diseases, in both pre- and post-harvest stages, among which gray mold rot, certain soil-borne fungi, and powdery mildew stand out in terms of economic impact (estimated at 13%, 10%, and 9% of the harvest, respectively) [1].

The ascomycete *Botrytis cinerea* is considered the most important postharvest pathogen in strawberry. It produces gray mold on fruit and senescing organs but also affects vegetative tissues [2]. Other relevant pathogens of the crop are those transmitted by the soil, such as the oomycete *Phytophthora cactorum* and the mitosporic fungus *Verticillium dahliae*. The

former causes crown and fruit rot, producing wilting, collapse, and death of the entire plant, and attacking the fruits, which appear tarnished. The latter is responsible for a vascular mycosis that manifests itself in a lack of growth or development and wilting of the oldest leaves, causing losses due to both death and weakening, resulting in lower production and fruit size.

To date, the most efficient approach to control strawberry phytopathogens is the application of conventional synthetic fungicides, with negative impacts on health and the environment [3] and risks associated with the development of resistant fungal strains [4]. Further, as of recently, in some European countries, fungicides are fully forbidden during the postharvest stage [5].

Apart from chemical control, other alternative methods to control strawberry phytopathogens include planting disease-resistant cultivars (although thus far cultivars highly resistant to strawberry grey mold have not been identified); changing cultivation methods; solarization and biofumigation with biocontrol agents; and the use of products of natural origin, such as plant extracts [6].

In line with this latter approach, the work presented herein explores the potential of extracts of *Uncaria tomentosa* for strawberry crop protection purposes. This tropical medicinal plant of the *Rubiaceae* family, popularly known as *cat's claw*, is native to the Amazon rainforest and other areas of Central and South America. It has been traditionally used to treat fever, asthma, abscesses, urinary infections, and wounds, and is effective as an anti-inflammatory, antioxidant, and antimicrobial agent [7].

According to Batiha et al. [8], *U. tomentosa* is rich in many phytoconstituents such as oxindole and indole alkaloids, glycosides, organic acids, proanthocyanidins, sterols, and triterpenes. Among the oxindole alkaloids, Bertol et al. [9] identified uncarine F, speciophylline, mitraphylline, isomitraphylline, rhynchophylline, isorhynchophylline pteropodine, and isopteropodine. Along with alkaloids, quinovic acid glycosides have also been characterized [10]. The highest contents of alkaloids and polyphenols have been found in the leaves, followed by those found in the stem bark and branches (Figure 1). Regarding quinovic acid glycosides, they have only been detected in significant amounts in the stem bark [11].

Figure 1. (**a**) Flowers, (**b**) stem with nail-shaped spines, (**c**) fruits, and (**d**) bark of *Uncaria tomentosa*.

Apart from the alkaloids, the bioactive properties of *Uncaria tomentosa* have also been attributed to its phenolic constituents. In the extracts of bark and leaves of *U. tomentosa*, Navarro-Hoyos et al. [12,13] identified hydroxycinnamic and hydroxybenzoic acids flavan-3-ols monomers, flavalignans-cinconines, procyanidin dimers, and trimers and propelargonidin dimers; and in leaves of *U. tomentosa*, procyanidins, propelargonidins, and mixtures of both, composed of (epi) catechin and (epi) afzelequin units [14]. Moreover, White et al. [15] isolated three flavonoids (artochamin C and the 5′-hydroxy cudraflavones A and B) that showed significant antibacterial activities against *Escherichia coli* ATCC 11775, *Staphylococcus aureus* ATCC 12600, *Klebsiella pneumoniae* ATCC 13883, and *Bacillus subtilis* ATCC 6051.

Encouraged by these results, in this work, we investigated the in vitro antimicrobial activity of the ammoniacal extract of cat's claw bark, alone and conjugated with chitosan oligomers (COS), against the three aforementioned phytopathogens. In addition, with the aim of improving the storage capacity of strawberries, the applicability of the COS-bark extract conjugate complexes for the sustainable postharvest control of gray mold was also explored in strawberry fruits artificially infected with *B. cinerea*.

2. Material and Methods

2.1. Plant Material and Reagents

The extract was prepared from a composite sample consisting of the bark of 10 *Uncaria tomentosa* specimens from La Merced, Chanchamayo, Peru. The bark samples, with a golden yellow (or light brown) color, were thoroughly mixed, dried, and reduced to a fine powder.

Strawberry fruits (*Fragaria* × *ananassa* cv. Fortuna) used in the postharvest protection studies were supplied by Sociedad Cooperativa Andaluza Santa María de La Rábida (Palos de la Frontera, Huelva, Spain) and had been cultivated according to organic farming regulations, with no use of synthetic pesticides. Fruits were harvested and immediately cold shipped so that experiments could be started within 24 h of harvesting. Fruits were selected on the basis of uniformity of size, absence of physical damage and fungal infection, and >75% of the surface showing red color, according to the criteria suggested by Romanazzi et al. [16].

High-molecular-weight chitosan (CAS 9012-76-4) was purchased from Hangzhou Simit Chem. and Tech. Co. (Hangzhou, China). Neutrase™ 0.8 L enzyme was supplied by Novozymes A/S (Bagsværd, Denmark). Octyl isobutirate (CAS 109-15-9) was acquired from Sigma-Aldrich Química (Madrid, Spain). Potato dextrose agar (PDA) was purchased from Becton Dickinson (Bergen County, NJ, USA).

Commercial fungicides used for comparison purposes, viz., Ortiva® (azoxystrobin 25%; reg. no. 22000; Syngenta), Vondozeb® (mancozeb 75%; reg. no. 18632; UPL Iberia), Armetil® 25 WP (metalaxyl 25%; reg. no. 25330; I.Q. Valles), and Fesil® (fosetyl-Al 80%, reg. no. 18795; Bayer) were kindly provided by the Plant Health and Certification Service (CSCV) of Gobierno de Aragón. These fungicides were selected either due to their weak risk of resistance, their favorable toxicological and/or environmental profile, or their significant pathogen control ability.

2.2. Phytopathogens Isolates

The fungal isolates of *B. cinerea* (code not available, but details on its provenance are provided in [17]), *P. cactorum* (CRD Prosp/59), and *V. dahliae* (MYC-1134) were supplied as subcultures in PDA by Richerd Breia and Hernâni Gerós from the Centre of Molecular and Environmental Biology (CBMA) at the University of Minho, by the Aldearrubia Regional Diagnostic Center (Junta de Castilla y León), and by the Center for Research and Agrifood Technology of Aragón (CITA), respectively.

2.3. Preparation of Bark Extracts, Chitosan Oligomers, and Conjugate Complexes

To dissolve the polyphenols and other bioactive compounds of interest contained in the bark of *U. tomentosa*, digestion in an aqueous ammonia solution was chosen. The bark extract was prepared according to the procedure described in [18] with modifications: the bark powder sample was first digested in an aqueous ammonia solution for 2 h, then sonicated in pulsed mode (with a 2 min stop every 2.5 min) for 10 min using a probe-type ultrasonicator (model UIP1000hdT; 1000 W, 20 kHz; Hielscher Ultrasonics, Teltow, Germany), and then allowed to stand for 24 h. It was then adjusted to neutral pH using acetic acid. Finally, the solution was centrifuged at 9000 rpm for 15 min, and the supernatant was filtered through Whatman No. 1 paper.

Chitosan oligomers with molecular weight <2000 Da were obtained following the procedure proposed by Santos-Moriano et al. [19], with the modifications described in [20].

The COS–bark extract and COS−*n*-octyl isobutyrate conjugate complexes were obtained by mixing the respective solutions in a 1:1 (*v*/*v*) ratio, followed by sonication for 15 min in five 3-minute pulses (so that the temperature did not exceed 60 °C). Infrared spectroscopy was used to confirm the formation of the conjugate complexes.

2.4. Characterization Procedures

The bark sample infrared spectrum was registered using a Thermo Scientific (Waltham, MA, USA) Nicolet iS50 Fourier transform infrared (FTIR) spectrometer, equipped with a diamond attenuated total reflection (ATR) system. The spectrum was collected over the 400–4000 cm^{-1} range with a 1 cm^{-1} spectral resolution, co-adding 64 scans.

The aqueous ammonia extract (neutralized with acetic acid) of the bark of *U. tomentosa* was analyzed by gas chromatography–mass spectrometry (GC–MS) at the Research Support Services (STI) at Universidad de Alicante (Alicante, Spain), using a gas chromatograph model 7890A coupled to a quadrupole mass spectrometer model 5975C (both from Agilent Technologies, Santa Clara, CA, USA). Chromatographic conditions: injection volume = 1 μL; injector temperature = 280 °C, in splitless mode; initial oven temperature = 60 °C, 2 min, followed by ramp of 10 $°C·min^{-1}$ up to a final temperature of 300 °C, 15 min. The chromatographic column used for the separation of the compounds was an Agilent Technologies HP-5MS UI of 30 m in length, 0.250 mm diameter, and 0.25 μm film. Mass spectrometer conditions: temperature of the electron impact source of the mass spectrometer = 230 °C and of the quadrupole = 150 °C; ionization energy = 70 eV. The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with the database of the National Institute of Standard and Technique (NIST11) and Adams [21].

2.5. In Vitro Antimicrobial Activity

The antimicrobial activity was investigated using the agar dilution method [22], incorporating aliquots of stock solutions into the PDA medium to provide final concentrations in the 62.5–1500 $μg·mL^{-1}$ range (although lower concentrations of 7.81, 15.62, 31.25, 39.05, 46.86, and 54.87 $μg·mL^{-1}$ were also assayed for the most effective treatments in order to obtain reliable PROBIT fittings). Mycelial plugs (ø = 5 mm) were transferred from the margin of seven-day-old fresh PDA cultures in the case of *B. cinerea* and two-week-old fresh PDA cultures in the case of *P. cactorum* and *V. dahliae* to plates filled with the amended media (three plates per treatment and concentration combination; each experiment was carried out twice). Plates containing only PDA medium were used as a control. Radial mycelium growth was determined by calculating the average of two perpendicular colony diameters for each replicate. After incubation in the dark at 25 °C for one week (*B. cinerea*) or two weeks (*P. cactorum* and *V. dahliae*), growth inhibition was calculated according to the formula: $((d_c - d_t)/d_c) \times 100$, where d_c is the average colony diameter in the control and d_t is the average diameter of the treated colony. The 50% and 90% effective concentrations (EC_{50} and EC_{90}, respectively) were estimated using PROBIT analysis in IBM SPSS Statistics v.25 software (IBM; Armonk, NY, USA). The level of interaction, i.e., the synergy factor (SF), was estimated according to Wadley's method [23].

2.6. Postharvest Protection Studies

The strawberries were superficially disinfected with a NaOCl 3% solution for 2 min, washed thrice with sterile distilled water, and dried in a laminar flow hood using sterile absorbent paper [24]. The strawberries were randomly distributed into four homogeneous groups of 45 fruits (i.e., three repetitions with 15 fruits per repetition and treatment), ensuring that all fruits were in the 20−30 g/fruit weight range and that they were larger than 25 mm in diameter. Three groups were treated with the formulation with the highest in vitro activity against *B. cinerea*, i.e., COS−*U. tomentosa*, at different concentrations (100, 500, or 1000 $μg·mL^{-1}$), while the fourth group was set as the control. The treatments consisted in immersing fruits for 5 min in sterile distilled water (control) or the COS−*U. tomentosa* solution, after which they were dried at room temperature in a laminar flow hood, using sterile absorbent paper.

Superficial wounds (ø = 5 mm) were made in the equatorial zone of each fruit, where a plug of *B. cinerea* PDA culture was placed (with the mycelium facing the fruit wound) [24]. Fruits were subsequently placed in covered plastic boxes and stored for 7 days at 4 °C,

95–98% RH, and then exposed to a 3-day shelf life at 20 °C, 95–98% RH, in agreement with Hernández-Muñoz et al. [25].

During storage, the percentage of rotten strawberries was recorded, as well as the severity of the disease according to an empirical scale with six degrees (0, healthy fruit; 1, 1–20% of infected fruit surface; 2, 21–40% of infected fruit surface; 3, 41–60% of infected fruit surface; 4, 61–80% of infected fruit surface; 5, more than 81% of the strawberry surface is infected and shows sporulation), according to Romanazzi et al. [16].

The SEM characterization of healthy and infected fruits was performed with a QUANTA 200FEG microscope (FEI, Oregon, United States). Operating conditions: 5 kV; 150–600× magnification. The micrographs were obtained under low-vacuum conditions with a large field detector (LFD).

2.7. Statistical Analysis

The results of the postharvest protection study were statistically analyzed in IBM SPSS Statistics v.25 software by analysis of variance (ANOVA), followed by a post hoc comparison of means by Tukey's test (because the requirements of homogeneity and homoscedasticity were met, according to the Shapiro–Wilk and Levene tests).

3. Results

3.1. Cat's Claw Bark Infrared Spectrum

The main infrared absorption bands present in the spectrum of *U. tomentosa* bark are summarized in Table 1, compatible with the presence of the functional groups of alkaloids, polyphenols, organic acid esters, and other phytoconstituents.

Table 1. Main absorption bands in the infrared spectrum of *U. tomentosa* bark.

Wavenumber (cm^{-1})	Assignment
3185	Bonded O-H stretching (cellulose, hemicellulose, lignin)
2922	–CH_2 asymmetric stretching of alkyls (cutine, wax, pectin)
2362	CN (alkaloids)
2343	C−H stretching
1653	O−H−O scissors-bending/C=N/amide
1560	aromatic C−H stretching/COO^- symmetric stretching (esters)
1520	Aromatic skeletal
1394	C−H bending
1258	Guaiacyl units
1046	C–O−H stretching/C–O deformation/O−H out-of-plane bending
992	CH_2 groups in cellulose
924	β-Glycosidic linkages (glucose units of cellulose chains)
870	Aromatic C−H stretching/methyl double bonds
817	C–C−H deformation
764	COO^- deformation (esters)/C−H aromatics

3.2. Cat's Claw Extract Constituents

Among the phytoconstituents identified by GC−MS (Table S1), the most important were (Figure 2): *n*-octyl isobutyrate (or caprylyl isobutyrate) (30.7%), methyl 19α-methyl-2-oxoformosanan-16-carboxylate (also called mitraphylline or rubradinin) (9.3%), tetrahydro-2-methylthiophene (4.7%), and α-methylmannofuranoside (4.4%).

3.3. Antimicrobial Activity

The results of mycelial growth inhibition tests against *B. cinerea*, *P. cactorum*, and *V. dahliae* of the ammoniacal extract of *U. tomentosa* bark and its main constituent (i.e., octyl isobutyrate), both alone and forming conjugate complexes with COS, are presented in Figures 3–5, respectively.

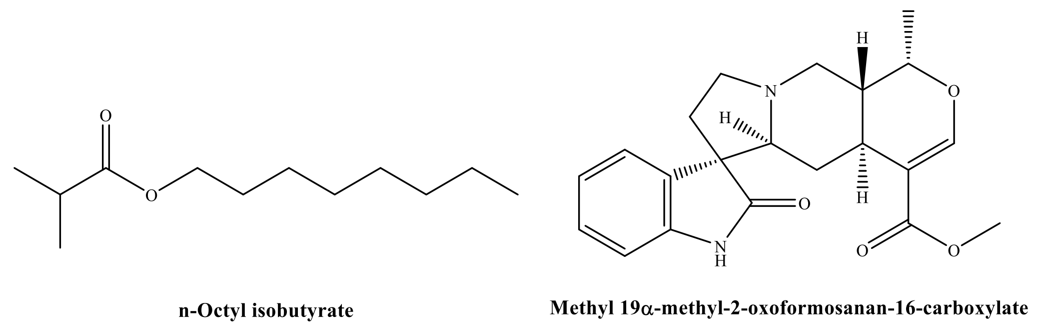

Figure 2. Main phytochemicals identified in *Uncaria tomentosa* bark extract.

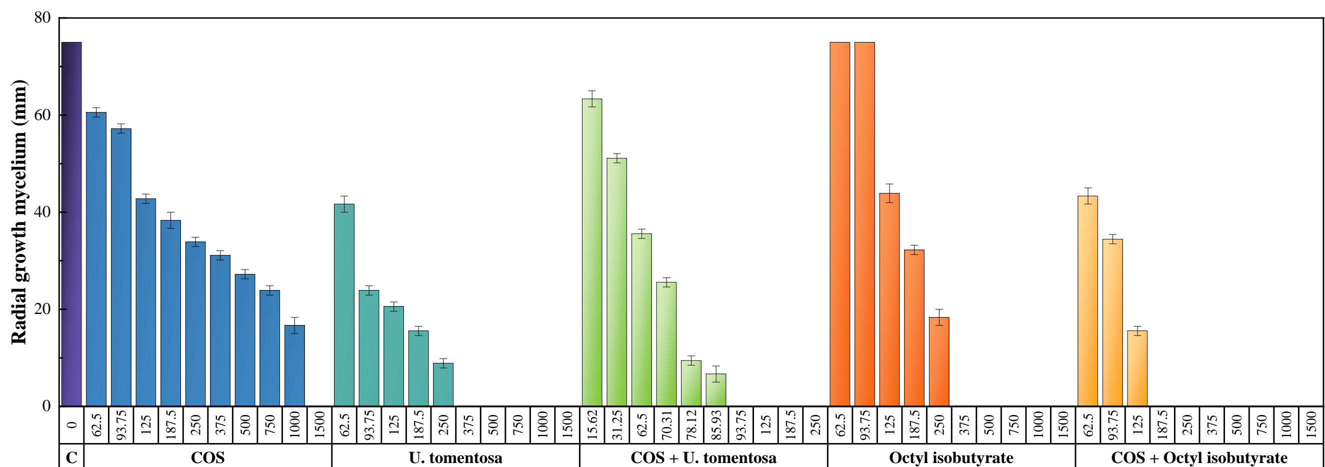

Figure 3. Inhibition of radial growth of the mycelium of *B. cinerea* in in vitro assays performed with different concentrations (in the 15.62–1500 μg·mL^{-1} range) of chitosan oligomers (COS), extract of *U. tomentosa* bark, the main constituent of the bark extract, and their respective conjugated complexes. Error bars represent standard deviations.

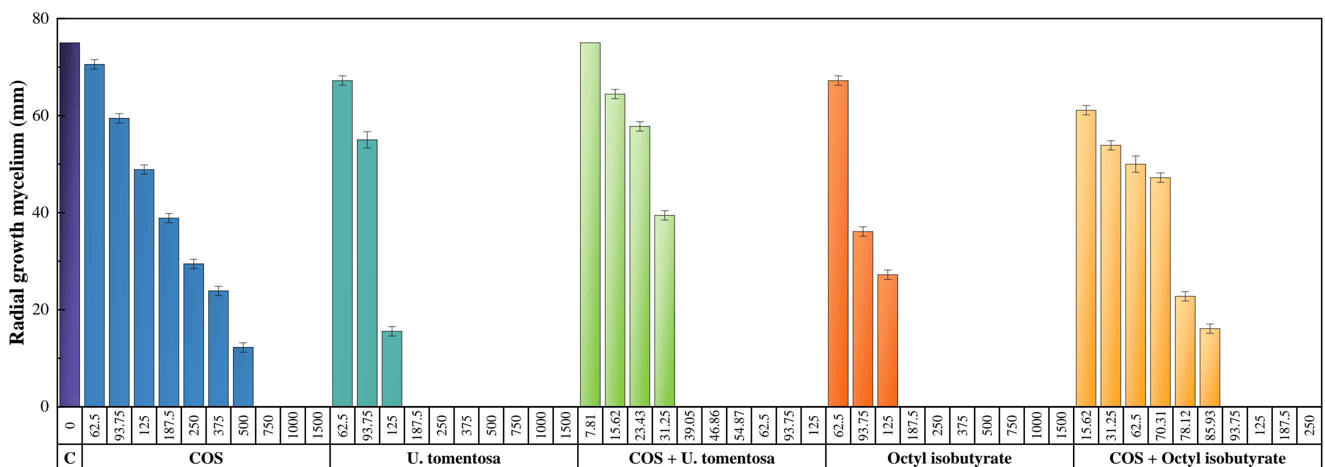

Figure 4. Inhibition of radial growth of the mycelium of *P. cactorum* in in vitro assays performed with different concentrations (in the 7.81–1500 μg·mL^{-1} range) of chitosan oligomers (COS), extract of *U. tomentosa* bark, the main constituent of the extract, and their respective conjugated complexes. Error bars represent standard deviations.

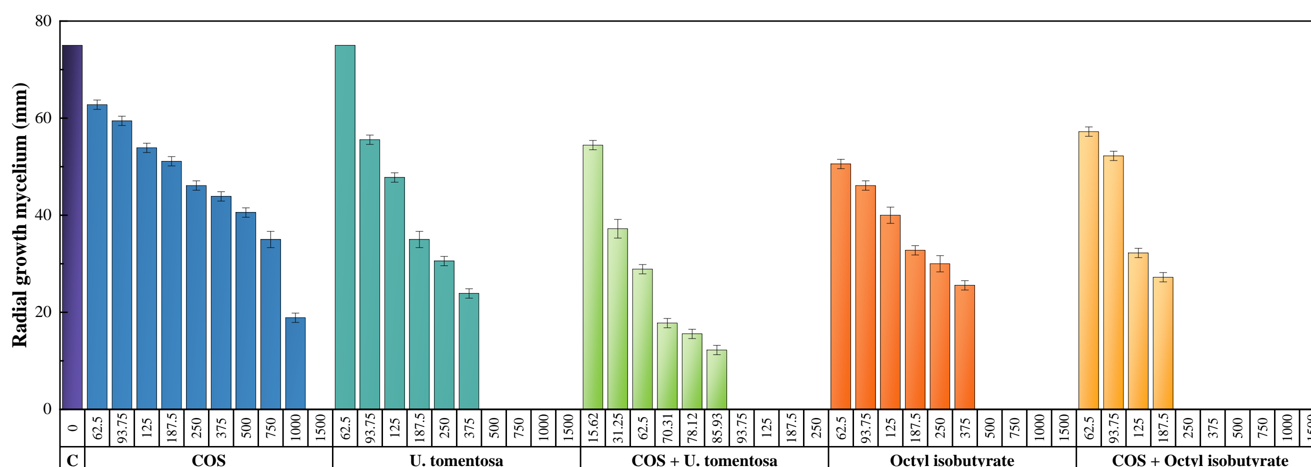

Figure 5. Inhibition of radial growth of the mycelium of *V. dahliae* in in vitro assays performed with different concentrations (in the 15.62–1500 μg·mL^{-1} range) of chitosan oligomers (COS), extract of *U. tomentosa* bark, the main constituent of the extract, and their respective conjugated complexes. Error bars represent standard deviations.

The antimicrobial activity of the bark extract upon conjugation with COS was found to be much higher than those of COS and bark extract alone, reaching full inhibition at concentrations in the 39−93.75 μg·mL^{-1} range, depending on the pathogen (vs. MICs in the 750−1500 and 187.5−500 μg·mL^{-1} range for COS and the pure bark extract, respectively).

Concerning the main bioactive compound found in *U. tomentosa* bark extract, pure octyl isobutyrate exhibited an antimicrobial activity comparable to that of the bark extract, with MICs in the 187.5−500 μg·mL^{-1} range. Even though its conjugation with COS also resulted in enhanced activity, reaching full inhibition at concentrations in the 93.75−250 μg·mL^{-1} range, its effectiveness was lower than that of the COS−bark extract conjugate.

To quantify the synergistic behavior observed for the conjugate complexes, effective concentrations were first estimated (Table 2) and synergy factors (SF) were then calculated according to Wadley's method (Table 3). The synergism between COS and *U. tomentosa* bark extract was noticeably higher than that observed between COS and octyl isobutyrate, with SF values in the 4.67−8.87 and 1.38−3.30 ranges, respectively.

Table 2. Effective concentrations (expressed in μg·mL^{-1}) against *B. cinerea*, *P. cactorum*, and *V. dahliae* of chitosan oligomers (COS), the ammoniacal extract of *U. tomentosa* bark, and octyl isobutyrate, alone and after conjugation.

Pathogen	COS		*U. tomentosa*		Octyl Isobutyrate		COS− *U. tomentosa*		COS−Octyl Isobutyrate	
	EC_{50}	EC_{90}	EC_{50}	EC_{90}	EC_{50}	EC_{90}	EC_{50}	EC_{90}	EC_{50}	EC_{90}
B. cinerea	236.2	1426.3	185.7	482.3	153.6	322.3	40.2	82.3	76.7	159.1
P. cactorum	200.8	592.8	103.3	162.8	91.5	171.9	29.2	38.3	73.6	89.3
V. dahliae	601.7	1321.2	185.7	482.3	142.4	471.2	32.0	87.5	116.5	248.9

Table 3. Synergy factors for the conjugate complexes estimated according to Wadley's method.

Pathogen	COS–*U. tomentosa*		COS–Octyl Isobutyrate	
	EC_{50}	EC_{90}	EC_{50}	EC_{90}
B. cinerea	5.17	8.76	2.43	3.30
P. cactorum	4.67	6.67	1.71	2.98
V. dahliae	8.87	8.08	1.38	1.92

For comparison purposes, the results of experiments conducted with four conventional synthetic fungicides are presented in Table 4. The highest effectiveness was recorded for a dithiocarbamate (mancozeb), finding full inhibition of the mycelial growth of the three phytopathogens at 1/10th of the recommended dose (i.e., at 150 μg·mL^{-1}), whereas the strobilurin fungicide (azoxystrobin) was the least effective, requiring up to 10 times the recommended dose (625 mg·mL^{-1}) to fully inhibit the growth of *B. cinerea* and *V. dahliae*. Concerning the other two fungicides tested, for which similar doses are recommended, the organophosphorus fungicide (fosetyl-Al) was more effective than the acylalanine one (metalaxyl) against *B. cinerea* and *V. dahliae*, but not against *P. cactorum* (for which full inhibition was reached at 210 μg·mL^{-1} in the case of metalaxyl).

Table 4. Radial growth of mycelium of *B. cinerea*, *P. cactorum*, and *V. dahliae* in in vitro assays performed on a PDA medium amended with different concentrations (the recommended dose, 1/10th of the recommended dose, and 10 times the recommended dose) of four commercial synthetic fungicides.

Commercial Fungicide	Pathogen	Radial Growth of Mycelium (mm)			Inhibition (%)		
		Rd/10	Rd *	Rd × 10	Rd/10	Rd *	Rd × 10
Azoxystrobin	*B. cinerea*	12	51	0	84	32	100
	P. cactorum	6	0	0	92	100	100
	V. dahliae	26	24	0	65.3	68	100
Mancozeb	*B. cinerea*	0	0	0	100	100	100
	P. cactorum	0	0	0	100	100	100
	V. dahliae	0	0	0	100	100	100
Metalaxyl	*B. cinerea*	45	21	0	40	72	100
	P. cactorum	0	0	0	100	100	100
	V. dahliae	41	36	0	45.3	52	100
Fosetyl-Al	*B. cinerea*	38	0	0	49.3	100	100
	P. cactorum	64	0	0	14.7	100	100
	V. dahliae	36	0	0	52	100	100

* Rd stands for recommended dose, i.e., 62.5 mg·mL^{-1} of azoxystrobin (250 g·L^{-1} for Ortiva®, azoxystrobin 25%), 1.5 mg·mL^{-1} of mancozeb (2 gL^{-1} for Vondozeb®, mancozeb 75%), 2.1 mg·mL^{-1} of metalaxyl (8.4 g·L^{-1} for Armetil 25 WP®, metalaxyl 25%), and 2 mg·mL^{-1} of fosetyl-Al (2.5 g·L^{-1} for Fesil®, fosetyl-Al 80%). The radial growth of the mycelium for the control (PDA) was 75 mm. All mycelial growth values (in mm) are average values (n = 3).

3.4. Postharvest Protection of Strawberry Fruits from Infection by B. cinerea

Strawberry fruits of "Fortuna" variety were treated with the most active product according to the in vitro tests, i.e., COS−*U. tomentosa* conjugate complex, at three different concentrations, corresponding to MIC, MIC×5, and MIC×10 (i.e., 100, 500, and 1000 μg·mL^{-1}, respectively). The greatest protection of strawberry fruits artificially inoculated with *B. cinerea* was observed at the highest concentration (1000 μg·mL^{-1}), with a degree of severity of only 0.5 according to the empirical scale proposed by Romanazzi et al. [16] (Table 5). This result indicates that no signs of infection (severity degree 0) were observed in half of the treated fruits and that the infection affected less than 20% of the total surface in the rest of the fruits (severity degree 1), vs. a degree of severity of 4.4 for the control (untreated) fruits, corresponding to around 70% of infected fruit surface (Figure 6). Visual observations were confirmed with SEM micrographs (Figure 7).

Table 5. Degree of severity of gray mold on strawberry fruits treated with COS−*U. tomentosa* conjugate complex 10 days after artificial inoculation with *B. cinerea*.

Coating	Severity (0−5)
Distilled water (control)	4.4 ± 0.7 [a]
COS−*U. tomentosa* 100 µg·mL^{-1}	3.5 ± 0.8 [b]
COS−*U. tomentosa* 500 µg·mL^{-1}	1.7 ± 0.8 [c]
COS−*U. tomentosa* 1000 µg·mL^{-1}	0.5 ± 0.5 [d]

Different letters indicate that the disease severity is significantly different at $p < 0.05$.

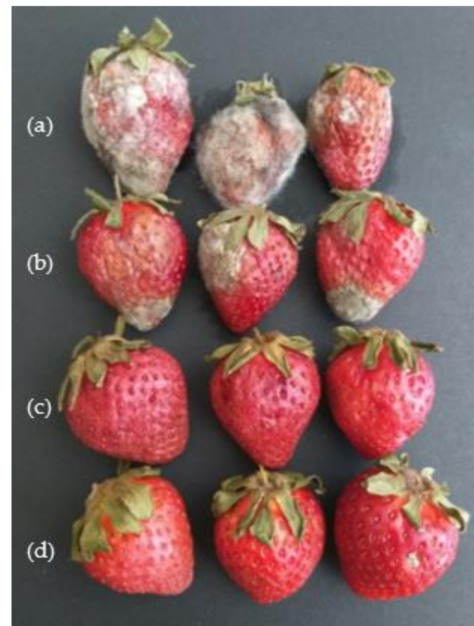

Figure 6. Decay by gray mold disease of strawberry fruits 10 days after artificial inoculation: (**a**) untreated fruits; fruits immersed in a COS−*U. tomentosa* solution at different concentrations, viz., (**b**) 100 µg·mL^{-1}, (**c**) 500 µg·mL^{-1}, and (**d**) 1000 µg·mL^{-1}.

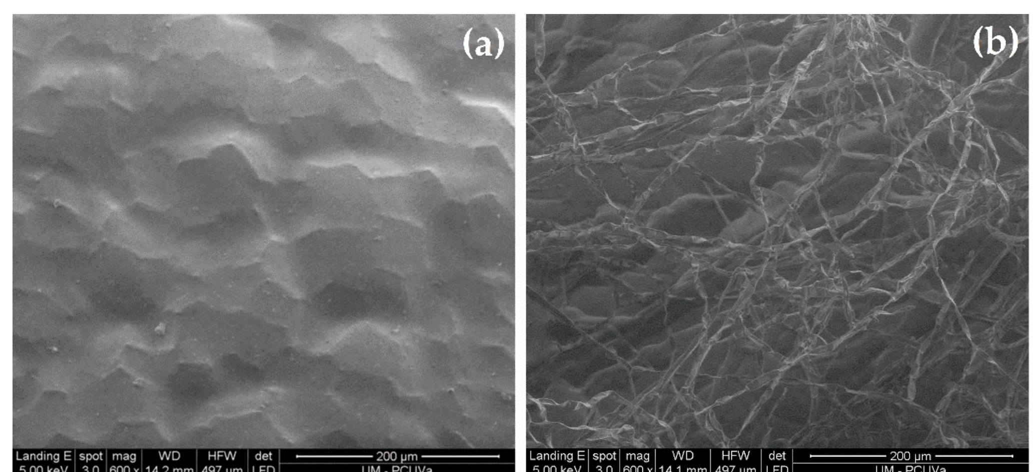

Figure 7. Scanning electron micrographs illustrating a comparison between strawberry fruits 10 days after artificial infection with *B. cinerea*: (**a**) treated fruits showed a smooth surface with no hyphal colonization, while (**b**) infected fruits showed the presence of numerous somatic hyphae on their surface.

4. Discussion

4.1. Comparison of In Vitro Activity

Octyl isobutyrate, the octyl ester of isobutyric acid (previously identified in *Mangifera indica* L. [26]), and mitraphylline, a pentacyclic oxindole (found in the leaves of *Mitragyna speciosa* (Korth.) Havil. [27] and in cat's claw bark along with several isomeric alkaloids [28]), were the two main phytocompounds present in the extract. Regarding the observed antimicrobial activity, in view of the results presented in Figure 3 (in which the effectiveness of the *U. tomentosa* bark extract and that of its main constituent were very similar), it may tentatively be mainly attributed to octyl isobutyrate. Nonetheless, a contribution of mitraphylline (the second major constituent) cannot be ruled out, given that both phytochemicals have been shown to feature antimicrobial activity in the literature [29–36] (see Table S2). In relation to the activity of COS, it is well established and may be ascribed to several mechanisms of action [37], including increased permeability of plasma membrane and the leakage of cellular contents, deprivation of trace elements essential for fungal normal growth due to its chelating action, and inhibition of mRNA synthesis and affection of protein and enzyme production due to binding to fungal DNA. As for the mechanism behind the high SFs observed for the conjugate complexes, synergism between COS and plant extracts may be explained by the fact that fungal pathogens are not resistant to multiple fungitoxicants [38], although solubility and bioavailability enhancements associated with conjugation have also been advocated in other works [39,40].

4.1.1. Comparison with Other Natural Compounds

Taking into consideration the fact that the susceptibility profile is isolate dependent, comparisons of the effective concentrations below should be taken with caution. Nonetheless, a bibliographic survey of natural products assayed against *B. cinerea*, *P. cactorum*, and *V. dahliae*, summarized in Table 6, indicates that the activity of the COS−*U. tomentosa* conjugate complexes (with MIC values in the 39−93.75 $\mu g \cdot mL^{-1}$ range) would be among the highest reported in the literature. In the case of *B. cinerea*, it would only be lower than that reported by Abou-Jawdah et al. [41] for an *Origanum syriacum* L. extract (60 $\mu g \cdot mL^{-1}$) and would be followed by those reported by Daferera et al. [42] for *Origanum vulgare* L., *Thymus capitatus* (L.) Hoffmanns. and Link, and *Origanum dictamnus* L. essential oils (with EC_{50} values in the 50−83 $\mu g \cdot mL^{-1}$ range vs. 40.2 $\mu g \cdot mL^{-1}$ for COS−*U. tomentosa*). As for *P. cactorum*, it would be similar to that of *Thymus serpyllum* L. essential oil (with an EC_{50} value of 20.45 $\mu g \cdot mL^{-1}$ [43], vs. 29.2 for COS−*U. tomentosa*, Table 2) and higher than that, for instance, of CUSTOS^{TM} formulated *Allium*-based extract (MIC = 100 $\mu g \cdot mL^{-1}$) [44]. Concerning *V. dahliae*, higher activities (MIC values ranging from 8 to 32 $\mu g \cdot mL^{-1}$) have been reported by Erdoğan et al. [45] for mint, thyme, and lavender essential oils (not for the plant extracts, with an effectiveness several orders of magnitude lower).

Table 6. Effective concentrations/MIC values reported in the literature for other bioactive natural products against the three pathogens under study.

Pathogen	Natural Product	Effective Concentration/MIC ($\mu g \cdot mL^{-1}$)	Ref.
B. cinerea	*U. tomentosa* bark extract	MIC = 375	This work
	COS−*U. tomentosa*	MIC = 93.75	
	Pimenta dioica PE	MIC = 2200	[46]
	Cinnamomum cassia PE	MIC = 600	
	Laurus nobilis PE	MIC = 3000	
	Syzygium aromaticum PE	MIC = 600	
	S. aromaticum EO	MIC = 1200	[47]
	L. nobilis PE	MIC > 2000	
	L. nobilis EO	MIC > 2000	
	Rosmarinus officinalis PE	MIC > 2000	
	R. officinalis EO	MIC > 2000	

Table 6. *Cont.*

Pathogen	Natural Product	Effective Concentration/MIC ($\mu g \cdot mL^{-1}$)	Ref.
	Anabaena sp.	MIC = 2500	[48]
	Ecklonia sp.	MIC = 5000	
	Jania sp.	MIC = 10,000	
	Achillea millefolium	MIC > 20,000	[49]
	Allium sativum	MIC = 20,000	
	Artemisia dracunculus	MIC > 20,000	
	Hyssopus officinalis	MIC < 5000	
	Mentha sp.	MIC = 20,000	
	R. officinalis	MIC > 20,000	
	Satureja hortensis	MIC = 10,000	
	Tagetes patula	MIC > 20,000	
	Valeriana officinalis	MIC > 20,000	
	Origanum vulgare EO	EC_{50} = 50	[42]
	Thymus capitatus EO	EC_{50} = 83	
	O. dictamnus EO	EC_{50} = 67	
	O. majorana EO	EC_{50} = 143	
	Lavandula angustifolia EO	EC_{50} = 223	
	R. officinalis EO	EC_{50} = 606	
	Salvia fruticosa EO	$EC_{50} \leq 1000$	
	M. pulegium EO	EC_{50} = 216	
	Micromeria nervosa PE	MIC = 500	[41]
	Origanum syriacum PE	MIC = 60	
	Inula viscosa PE	MIC > 2×10^6	
	Plumbago maritime PE	MIC = 1×10^6	
	O. heracleoticum PE	MIC > 5×10^5	[50]
	Salvia officinalis PE	MIC > 5×10^5	
	R. officinalis PE	MIC > 5×10^5	
	Pinus sylvestris bark	MIC = 20,000	[51]
	P. abies bark	MIC = 20,000	
	Liquidambar orientalis PE	MIC > 4×10^5	[52]
	Myrtus communis PE	MIC = 400	
P. cactorum	*U. tomentosa* bark extract	MIC = 187.5	This work
	COS−*U. tomentosa*	MIC = 39.05	
	Allium-based extract	MIC = 100	[44]
	O. heracleoticum PE	MIC > 5×10^5	[50]
	S. officinalis PE	MIC > 5×10^5	
	R. officinalis PE	MIC > 5×10^5	
	P. sylvestris bark	MIC = 100	[51]
	P. abies bark	MIC = 100	
	T. serpyllum EO	EC_{50} = 20.45	[43]
	Eucalyptus citriodora EO	MIC > 28,000	[53]
	Melaleuca quinquenervia EO	MIC > 28,000	
	Leptospermum pertersonii EO	MIC = 28,000	
	Polylepis. racemosa EO	MIC > 28,000	[54]
	Junierus oxycedrus EO	MIC > 28,000	
	Cymbopogon nardus EO	MIC > 28,000	
	Pelargonium graveolens EO	MIC = 28,000	
	Cuminum cyminum EO	MIC > 28,000	
	Myrristica fragrans EO	MIC > 28,000	
	C. martini EO	MIC = 28,000	
	M. pulegium EO	n.a.	
	M. spicata EO	n.a.	
	T. vulgaris EO	MIC = 14,000	

Table 6. *Cont.*

Pathogen	Natural Product	Effective Concentration/MIC ($\mu g \cdot mL^{-1}$)	Ref.
V. dahliae	*U. tomentosa* bark extract	MIC = 500	This work
	COS−*U. tomentosa*	MIC = 93.75	
	O. heracleoticum PE	MIC > 5 × 10^5	[50]
	S. officinalis PE	MIC > 5 × 10^5	
	R. officinalis PE	MIC > 5 × 10^5	
	Propolis	MIC > 60,000	[55]
	M. piperita EO/PE	MIC = 16 / > 1 × 10^5	[45]
	T. vulgaris EO/PE	MIC = 8 / > 1 × 10^5	
	Lavandula angustufolia EO/PE	MIC = 32 / > 1 × 10^5	

PE = plant extract; EO = essential oil; n.a. = no activity.

4.1.2. Comparison with Conventional Fungicides

Considering the mycelial growth inhibition recorded for the four selected conventional fungicides (Table 4), the antimicrobial activity of the COS−*U. tomentosa* conjugate complex (93.75, 39.05, and 93.75 $\mu g \cdot mL^{-1}$ against *B. cinerea*, *P. cactorum*, and *V. dahliae*, respectively) would be much higher than those of azoxystrobin (with MICs >62.5, >6.25, and >62.5 $mg \cdot mL^{-1}$, respectively), and substantially higher than those of metalaxyl (with MICs of >2.1, >0.21, and >2.1 $mg \cdot mL^{-1}$, respectively) and fosetyl-Al (>200 $\mu g \cdot mL^{-1}$ against the three pathogens). Hence, it would be comparable to that of mancozeb, for which full inhibition was attained at concentrations below 150 $\mu g \cdot mL^{-1}$.

If the activity is instead compared with those reported for the same four fungicides in the literature (Table 7), remarkable differences in the reported MICs are observed (vs. this work and among works by different authors), tentatively ascribed to the use of very different (in terms of host range, aggressivity, and virulence) isolates from various crops. Still, metalaxyl and mancozeb would be the most effective, with, for instance, MIC values $\leq$100 $\mu g \cdot mL^{-1}$ against *P. cactorum* (comparable to the results reported in Table 4 and discussed in previous paragraph). However, their activity would be lower than that of the COS−*U. tomentosa* conjugate complex.

Table 7. Effective concentrations/MIC values reported in the literature for four conventional fungicides against the three pathogens under study.

Synthetic Fungicide	Pathogen	Provenance of Isolate	Effective Concentration/MIC ($\mu g \cdot mL^{-1}$)	Ref.
Azoxystrobin	*B. cinerea*	Strawberry	$EC_{50} \geq 100$	[56]
			$EC_{50} \geq 71.9$	[57]
		Grapevine	$EC_{50} \geq 50$	[58]
	P. cactorum	Strawberry	MIC $\geq$ 100	[59]
	V. dahliae	Avocado tree	MIC $\geq$ 40,000	[60]
		Olive tree	MIC = 1000	[61]
		Pepper	EC_{50}= 71.95	[62]
Mancozeb	*P. cactorum*	Apple tree	MIC = 100	[63]
		Strawberry	MIC = 100	[59]
Metalaxyl	*P. cactorum*	Apple tree	MIC = 50	[63]
		Peach tree	MIC = 1 × 10^5	[64]
		Strawberry	MIC = 100	[59]
Fosetyl-Al	*P. cactorum*	Apple tree	MIC = 1000	[63]
		Peach tree	MIC = 1.5 × 10^6	[64]
	V. dahliae	Olive tree	MIC = 5000	[61]

On the other hand, in terms of cost, the treatment reported herein would be more expensive than the synthetic fungicides. The price of cat's claw bark in natural product wholesalers is approximately EUR 25/kg, out of which 720 g of extract (lyophilized) may be obtained, so the final price of the extract turned out to be EUR 35/kg. Concerning chitosan, the wholesale price is approximately EUR 40/kg, which increases to EUR 55/kg due to COS preparation costs. Hence, the cost of the reagents to prepare the conjugate complexes (in a 1:1 ratio) would be in the EUR 45–50/kg range (vs. EUR 8/kg for mancozeb 75–80% formulates). In this way, 1 L of a 1000 $\mu g \cdot mL^{-1}$ conjugate complex solution would cost around EUR 0.05, which would be affordable, for instance, for organic strawberry production.

4.2. Comparison of Effectiveness of Postharvest Treatment

A summary of treatments with chitosan and other alternatives to conventional fungicides to control gray mold decay of strawberries reported in the literature is presented in Table 8. It may be observed that the effectiveness of the COS−*U. tomentosa* treatment was remarkably higher than those reported for chitosan acetate, chloride, glutamate, and formate, or chitosan oligosaccharides [16]. Even the most effective product reported to date, consisting of *Zataria multiflora* Boiss. essential oil encapsulated in chitosan nanoparticles (1500 $\mu g \cdot mL^{-1}$), resulted in lower protection [65], given that the decay severity was comparable to that attained in this work with COS−*U. tomentosa* at 500 $\mu g \cdot mL^{-1}$.

Table 8. Treatments with chitosan and other alternatives to conventional fungicides to control gray mold decay of strawberry reported in the literature and their associated decay severity.

Application	Natural Product	Storage Conditions	Severity (0–5)	Ref.
Postharvest	Chitosan acetate (1% *w*/*v*)	4 days at 20 ± 1 °C, 95–98% RH	3.1	[16]
	Chitosan chloride (1% *w*/*v*)		3.2	
	Chitosan formate (1% *w*/*v*)		3.4	
	Chitosan glutamate (1% *w*/*v*)		3.4	
	Commercial chitosan (1% *w*/*v*)		3.5	
	Abies sibirica extract (1% *v*/*v*)	7 days at 0 ± 1 °C, 95–98% RH, followed by 3 days of shelf life at 20 ± 1 °C, 95–98% RH	2.2	
	Oligosaccharides (1% *v*/*v*)		3.4	
	Chitosan (1% *w*/*v*)		2.7	
	Ca+organic acids (1% *v*/*v*)		3.4	
	Urtica dioica extract (1% *w*/*v*)		2.9	
	Soybean lecitin (1% *v*/*v*)		3.2	
	Chitosan NP (1500 $\mu g \cdot mL^{-1}$)	7 days at 4 °C, followed by 2 days at 20 °C	2.6	[65]
	Chitosan NP-*Zataria multiflora* (1500 $\mu g \cdot mL^{-1}$)		1.5	
	Cinnamomum zeylanicum EO (1500 $\mu g \cdot mL^{-1}$)		3.2	[67]
	Zataria multiflora EO (1500 $\mu g \cdot mL^{-1}$)		3.5	
	Chitosan + *C. zeylanicum* (1500 $\mu g \cdot mL^{-1}$)		2.4	
	Chitosan + *Z. multiflora* (1500 $\mu g \cdot mL^{-1}$)		1.5	
Preharvest	Chitosan 0.5%	7 days at 0.5 ± 1 °C, followed by 4 days at 20 ± 1 °C and 95–98% RH	2.1−3.0 *	[66]
	Chitosan 1%		2.0−2.8 *	
	Fir extract/organic acids and Ca (10 $mg \cdot mL^{-1}$)		2.0−3.5 *	
	Laminarin 1%		2.3−3.3 *	
	Laminarin + *Saccharomyces* spp. extract (1 + 3 $mg \cdot mL^{-1}$)		2.0−3.1 *	
	Laminarin + *Polygonum* spp. extract (1 + 3 $mg \cdot mL^{-1}$)		1.8−3.0 *	

* Depending on strawberry cultivar and season. NP stands for nanoparticles.

It is worth noting that when analogous chitosan-based treatments were applied in field conditions (preharvest applications) [66], similar or slightly higher postharvest protection was attained than for postharvest treatments. Hence, high protection may be also expected if the proposed COS−*U. tomentosa* treatment is sprayed over the strawberry plants during the growing season (although field experiments would be needed to confirm this point in order to optimize the dosage and number of applications, as well as to exclude phytotoxicity).

5. Conclusions

The aqueous ammonia extract of *Uncaria tomentosa* bark, rich in octyl isobutyrate (30.7%) and mitraphylline (9.3%), was first tested in vitro against *B. cinerea*, *P. cactorum*, and *V. dahliae*, alone and in combination with chitosan oligomers, resulting in full inhibition at concentrations in the 187.5−500 and 39−93.75 $\mu g \cdot mL^{-1}$ range, respectively, depending on the pathogen assayed, evidencing a strong synergism between COS and the bark extract (SF = 4.67−8.87). This effectiveness was among the highest reported in the literature for other bioactive natural products against the aforementioned strawberry phytopathogens and was also higher than those found when four conventional synthetic fungicides (azoxystrobin, mancozeb, metalaxyl, and fosetyl-Al) were applied. In view of its enhanced activity, the COS−*U. tomentosa* conjugate complex was further tested as a protective agent to prevent gray mold decay during strawberry (cv. Fortuna) fruit cold storage, finding that immersion in a 1000 $\mu g \cdot mL^{-1}$ conjugate complex solution for 5 min resulted in a severity decay of 0.5 (vs. 4.4 for the control fruits). Such protective activity was substantially higher than those reported for other chitosan or chitosan–plant derivative coatings. Hence, these findings suggest that cat claw's bark extracts may be a promising source of antimicrobials for agriculture and, in particular, an alternative to conventional synthetic fungicides for the pre- and post-harvest control of phytopathogens of strawberry crops.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae8080672/s1, Table S1. Phytochemicals identified by GC−MS in the ammoniacal extract of *U. tomentosa* bark. Table S2. Antimicrobial activity reported in the literature for other natural products rich in octyl isobutyrate or mitraphylline.

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