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NASKO HRISTOV NACHEV

NOVEL ECO-FRIENDLY ELECTROSPUN POLYMER MATERIALS WITH ANTIFUNGAL ACTIVITY

DISSERTATION ABSTRACT

presented for acquisition of
the Educational and scientific degree "Doctor"

Sofia, 2022

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Professional field: 4.2. Chemical Sciences

Specialty: Polymers and Polymer materials

Scientific supervisors:

Prof. Iliya Rashkov, DSc., Corr. Member of BAS

Assoc. Prof. Mariya Spasova-Todorova, PhD

Sofia, 2022

The dissertation was discussed and admitted to defense at a meeting of the Colloquium of the Institute of Polymers - BAS

The dissertation is presented on 115 pages, and includes 45 figures. 257 references are used. The results are reported in 3 publications and 9 science forums.

The defense of the dissertation will take place on .....2022 at ……..h in the meeting room of the Institute of Polymers - BAS at a meeting of the scientific jury.

The defense materials are available to the interested ones in the office of the Institute of Polymers - BAS; Sofia, Akad. G. Bonchev St., Bl. 103B

Author: Nasko Hristov Nachev

Dissertation title: NOVEL ECO-FRIENDLY ELECTROSPUN POLYMER MATERIALS WITH ANTIFUNGAL ACTIVITY

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These studies are part of the research performed at LBAP, in the field of novel polymeric materials with fungicidal activity, obtained by the electrospinning method, intended for application in agriculture.

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I would like to thank from the bottom of my heart to Prof. Iliya Rashkov, DSc, Corresponding Member of BAS, and Prof. Nevenka Manolova, DSc and Assoc. Prof. Mariya Spasova-Todorova, PhD for their faith in me, for providing me with the valuable guidance and counsel, for the new knowledge gained and the endless support for the development and design of the dissertation. I highly appreciate the comprehensive help, understanding, patience, good and friendly attitude.

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The studies summarized in the dissertation are performed in the Laboratory of Bioactive Polymers, Scientific field “Polimeric biomaterials”, Institute of Polymers, Bulgarian Academy of Sciences (IP-BAS), with the Head Prof.Dr.Olya Stoilova. Part of the research was carried out in regard to the implementation of Grant KP-06-OPR03/2, financed by the Bulgarian National Science Fund. The research is part of systematic research in the field of innovative electrospun micro- and nanocomposite fibers with potential applications for agropharmaceuticals (APS).

LIST OF ABBREVIATIONS

5-Cl8Q – 5-chloro-8-hydroxyquinoline
AFM - atomic force microscopy
CA - cellulose acetate
CQ - 5-chloro-7-iodo-8-hydroxyquinoline
DCM - dichloromethane
DMF - dimethylformamide
DSC - - differential scanning calorimetry
EtOH - ethanol
FTIR - Fourier transform infrared spectroscopy
K5N8Q - potassium 5-nitro-8-quinolinolate
P. aleophilum - *Phaeoacremonium aleophilum*
P. chlamydospora - *Phaeomoniella chlamydospora*
PCL - poly(ε-caprolactone)
PEG - poly(ethylene glycol)
PEO - poly(ethylene oxide)
PHA - polyhydroxyalkanoates
PHB - poly(3-hydroxybutyrate)
PLA - polylactide
PLLA - poly(L-lactide)
PP - polypropylene
PVA – poly(vinyl alcohol)
PVP - polyvinylpyrrolidone
T. viride - *Trichoderma viride*
UV-VIS - Ultraviolet–visible spectroscopy

XPS - X-ray photoelectron spectroscopy
XRD - X-ray structural analysis
APS - agro-pharmaceuticals
BAS - biologically active substance
MIC - minimum inhibitory concentration
SEM - scanning electron microscopy
TEM - transmission electron microscopy

The research literature (references) cited in the dissertation is indicated by first author and year of publication. The scientific publications presented in the dissertation are given in brackets as an upper index to each chapter. The numbers of the designations correspond to the numbering in the list of publications and scientific communications of the doctoral student, attached at the end of the dissertation.

[P №] - indicates the PhD student's publication;
[PP №] - indicates a scientific communication in the form of a poster presentation with the participation of the PhD student;
[OP №] – indicates a scientific communication in the form of an oral presentation with the participation of the PhD student.

The numbering of sections and figures in the dissertation abstract corresponds to that in the dissertation.

I. INTRODUCTION

In the last 15-20 years, considerable interest has been focused on innovative electrokinetic technologies. These include polymer melt electrospinning or solution electrospinning, electrospraying, centrifugal electrospinning, coaxial electrospinning etc. The so-called non-woven fabric, which is characterized by high porosity and its possibilities for the most diverse applications, is able to be fabricated by using these electrokinetic methods for preparation of micro- and nanofibrous materials. These innovative methods allow fibrous materials from both synthetic and natural biocompatible and biodegradable polymers, harmless to the environment to be obtained. It is possible some low molecular weight compounds with biological activity (antimicrobial or antifungal), different in their chemical nature and composition to be included in these micro- or nanofibrous materials. Since 2002, the Laboratory of Bioactive Polymers has been carrying out systematic research in the field of preparation of micro- and nanofibrous polymer materials with biological activity imparted. Systematic research is also being conducted to improve these electrokinetic methods for obtaining fibrous materials. The created equipment allows obtaining fibers with different designs, where the biologically active compound can be located "*in*" the fibers or "*on*" the surface of the fibers (electrospinning/electrospraying) upon the production of innovative plant protection materials.

The application of innovative nanotechnologies for the fabrication of plant protection materials is an extremely promising new direction, that mostly uses the application of environmentally friendly and biodegradable polymers. For more than 20 years, the application of such polymers has been a main research direction of the collaborators from the Laboratory of Bioactive Polymers. Cellulose acetate, poly(L-lactic acid) and its copolymers, chitosan and others take place among the studied biocompatible and/or biodegradable polymeric materials. A modern trend in the development of innovative plant protection materials is the use of polymer materials with their own biological activity, such as chitosan, as well as the use of synthetic biologically active, low-molecular compounds, metal oxides or useful soil microorganisms.

Esca disease is one of the earliest known diseases of grapevines. It is widespread in all wine-growing countries and occurs throughout the world, causing huge economic losses. In the last three decades, the attention to esca has been extremely intensified worldwide - the cases of manifestation of the disease have increased significantly. The disease spreads rapidly and poses a real threat to the vineyards in Europe. In Bulgaria, until the 90s of the 20th century, it was found mainly in old vineyards and was able to destroy up to 30% of the vines. At the beginning of the 21st century, however, esca was also observed in young 4-5 year old plantations.

The disease-causing agents are fungi, most often of the species *P. chlamydospora* and *P. aleophilum*. The wounds obtained during the pruning of the vines are considered the "main entrance" for the penetration of the fungal spores into the plant.

Sodium arsenite has been used to treat and prevent the disease. In 2003, sodium arsenite was recognized as highly toxic and carcinogenic and has been banned for agricultural use in Europe ever since. Nowadays, there is practically no curative approach to combating esca. Therefore, it is imperative to find new approaches and protection materials against esca.

For the first time in 2015, Sett and co-authors reported the fabrication of silk membranes electrospun with soy protein/PVA and soy protein/PCL nanofibers used for plant protection. This material is intended to physically block the penetration of fungal spores. However, the authors are of the opinion that blocking does not occur to a sufficient extent and there is a need to include an antifungal component.

Electrospun materials of poly(lactide-*co*-glycolide) and poly(butadiene adipate-*co*-terephthalate) incorporated with polyhexamethyleneguanidine to inhibit the penetration of *P. chlamydospora* spores have also been obtained. Electrospun mats can be directly attached to the pruning wound and thus provide a barrier against the penetration of fungal spores, while not impeding the entry of water and air to the plant. The authors of this work reveal, however, that there is a need to search for more effective and sustainable antifungal additives and a better selection of the polymers used.

In research carried out at LBAP, a method for easy preparation of fibrous membranes based on cellulose acetate and PEG, containing a derivative of 8-hydroxyquinoline for active protection against spore penetration and protection of grapevines from the esca disease was proposed. The resulting novel fibrous

materials completely suppress the growth of the fungal strains that cause the disease esca.

Environmentally friendly materials based on PHB, nanosized TiO_2 – anatase and chitosan oligomers have also been successfully obtained by simultaneous electrospinning and electrospraying. Decorating the PHB fibers with TiO_2 resulted in materials that inhibited the growth of *P. chlamydospora*.

The studies in the current dissertation are aimed at obtaining new eco-friendly electrospun polymer materials with fungicidal activity. The possibilities for electrospinning solutions of polyester - PLLA, polyhydroxyalkanoate - PHB and 8-hydroxyquinoline derivatives, as well as electrospinning of polysaccharide dispersions - CA and ZnO nanoparticles were investigated.

II. AIM AND OBJECTIVES OF THE DISSERTATION

The aim of the present dissertation work is the preparation of novel eco-friendly polymer materials with antifungal activity by electrospinning/electrospraying processes and the complex characterization and clarification of the potential possibilities for their application for agricultural purposes and, more specifically, to protect grapevines from penetration and infection with fungal spores, causative agents of the disease esca.

Upon the development of the dissertation work with regard to this aim, he following tasks were formed:

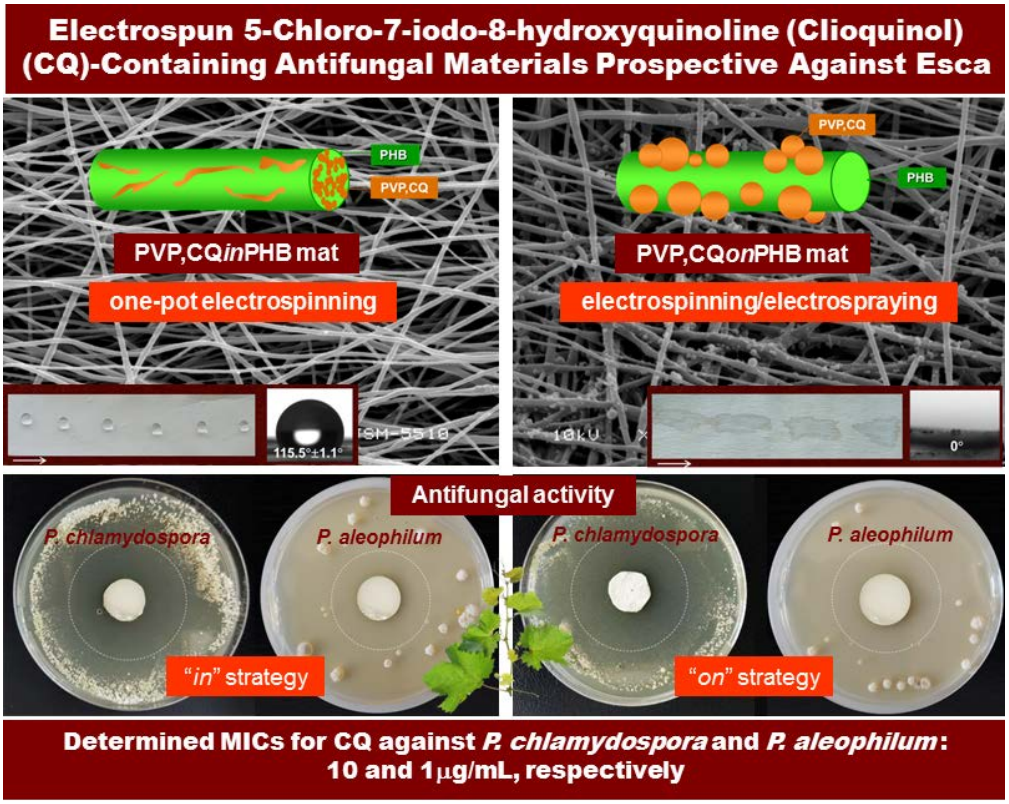
1. Fabrication of novel fibrous materials from PHB, PVP and 5-chloro-7-iodo-8-hydroxyquinoline (clioquinol) (CQ) by electrospinning and electrospraying.
2. Investigation of the CQ *in vitro* release profile based on the design of the electrospun materials and the techniques of their preparation.
3. Preparation and characterization of new electrospun polymer composites based on PLLA and various 8-hydroxyquinoline derivatives.
4. Investigation of the antifungal activity of PLLA/K5N8Q and PLLA/5-Cl8Q against the fungal strains *P. chlamydospora* and *P. aleophilum.*
5. Preparation of novel cellulose acetate-based fibrous materials decorated with ZnO nanoparticles by electrospinning and their characterization.
6. Study and improvement of the water-repellent and antifungal properties of the obtained innovative CA/ZnO materials.

7. Investigation of the potential of the obtained new materials to as active coatings for the protection of grapevine plants from the esca disease.

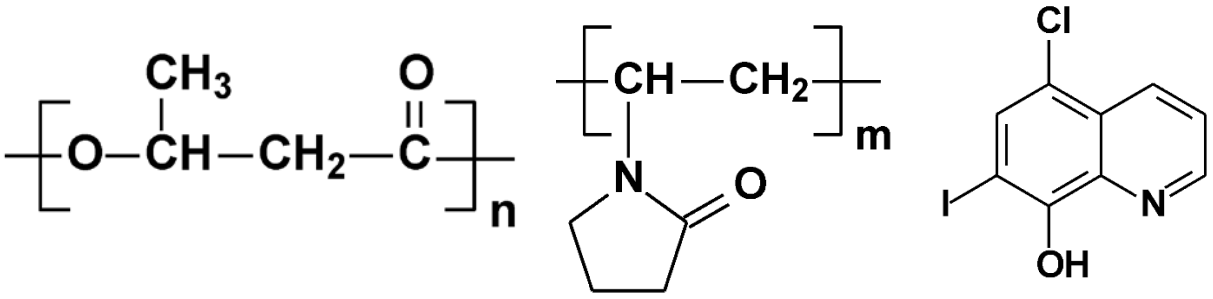
III. RESULTS AND DISCUSSION

Chapter 1. ELECTROSPUN CQ-CONTAINING POLY(3-HYDROXYBUTYRATE)/POLYVINYLPYRROLIDONE - BASED ANTIFUNGAL MATERIALS FOR THE PROTECTION OF VINEYARDS AGAINST ESCA DISEASE

[P 3] [PP 4]



This Chapter summarizes the results on the preparation and characterization of new fibrous materials with antifungal properties based on poly(3-hydroxybutyrate) (PHB), polyvinylpyrrolidone (PVP) and 5-chloro-7-iodo-8-hydroxyquinoline (CQ) (Figure C1-1). One-pot electrospinning ("in" strategy) or electrospinning in conjunction with electrospraying ("on" strategy) were applied to obtain the materials. The materials` morphology and their surface chemical composition were examined using scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS) and Fourier-transform infrared spectroscopy. CQ incorporated in the bulk of the fibers or in PVP particles deposited on the fibers was in the amorphous phase, which was confirmed by differential scanning calorimetry (DSC) and X-ray diffraction analysis (XRD). The in vitro release of CQ depended on the composition of the electrospun materials and on their design and a microbiological screening against the fungi *P. chlamydospora* and *P. aleophilum* was also performed.



A **B** **C**

Figure C1-1. Structure of: A. poly(3-hydroxybutyrate) (PHB), B. polyvinylpyrrolidone (PVP) and C. 5-chloro-7-iodo-8-hydroxyquinoline (5Cl7I8Q))

1.1. Preparation and morphology of fibrous materials based on PHB, PVP and CQ

Polyvinylpyrrolidone can plasticize certain polymers, as well as forming hydrogen bonds to help release some biologically active compounds from micro- and nanofibrous materials.

The combining of the beneficial properties of the aliphatic polyester PHB and of water-soluble polymer PVP with the antifungal properties of CQ is a favorable strategy for the fabrication of novel fibrous materials appropriate for diverse applications in agriculture.

In this study, for the fabrication of fibrous materials from PHB and PVP containing CQ of diverse design, two different approaches were developed: one-step electrospinning of fibers from a solution of PHB, PVP and CQ ("*in*" strategy, Figure C1-2A) and electrospinning of a PHB solution in conjunction with electrospraying of a PVP,CQ solution ("*on*" strategy, Figure C1-2B).

The electrospinning of solutions of PHB, PVP and CQ in a CHCl3/DMF solvent system (4/1 v/v) at a total polymer concentration of 10 wt % led to the formation of cylindrical and defect-free fibers (Figure C1-3B). PHB (Figure C1-3C) and PVP*in*PHB fibers (90:10 w/w) (Figure C1-3A) were also obtained using the selected conditions. The mean diameter of the PHB fibers was 760 ± 200 nm. The addition of PVP to the PHB solutions imparted a decrease in the average fiber diameters to 480 ± 110 nm (Figure C1-3 A, C). This effect is most likely due to a decrease in the viscosity of the PHB solution from 180 cP to 110 cP with the addition of PVP. When CQ (10 wt%) was added to the PHB/PVP solution, the average fiber diameter changed only negligible (Figure C1-3A) - from 480 ± 110 nm for the PVP*in*PHB (Figure C1-3A) mat to 470 ± 110 for the PVP,CQinPHB mat (Figure C1-3B). This might be attributed to

the slight decrease in the viscosity of the PHB/PVP solution from 110 cP to 100 cP on adding of CQ to the solution.

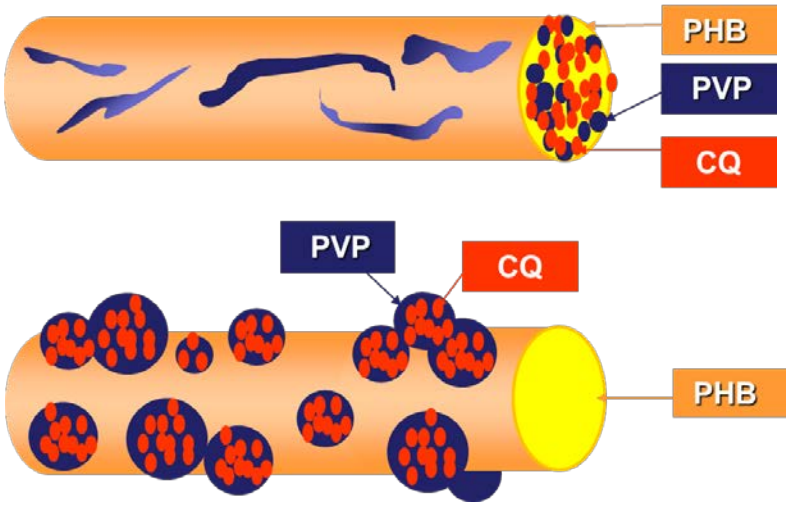
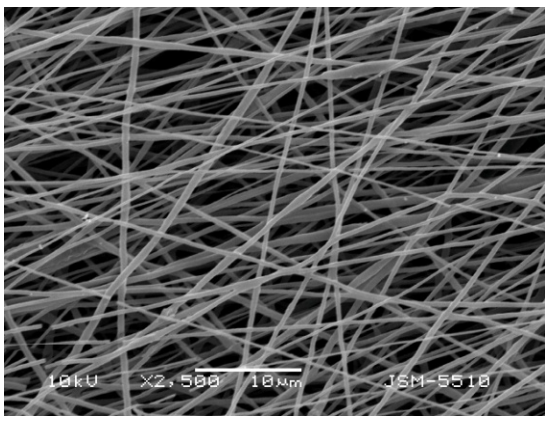


Figure C1-2. Schematic representation of fibers: A. PVPinPHB fiber loaded with CQ in the bulk (PVP,CQinPHB) and B. PHB fiber decorated with PVP,CQ particles on the surface (PVP,CQonPHB).

PHB fibers decorated with PVP,CQ particles were obtained by performing simultaneous electrospinning and electrospraying. SEM micrographs of PVP,CQ*on*PHB mats are presented in Figure C1-3D. As can be seen, particles of CQ and PVP were deposited on the surface of PHB fibers. SEM micrographs show that a significant part of the obtained particles are located on the PHB fibers, another part of the particles are located in the space between the fibers or in the lower layers forming the mat. It is evident that the particles of PVP,CQ deposited on the PHB fibers had spherical shape Figure C1-3D). The average particle size for the PVP,CQ*on*PHB mats was 490 ± 150 nm and 1050±130 nm for the small and large particles, respectively (Figure C1-3D).



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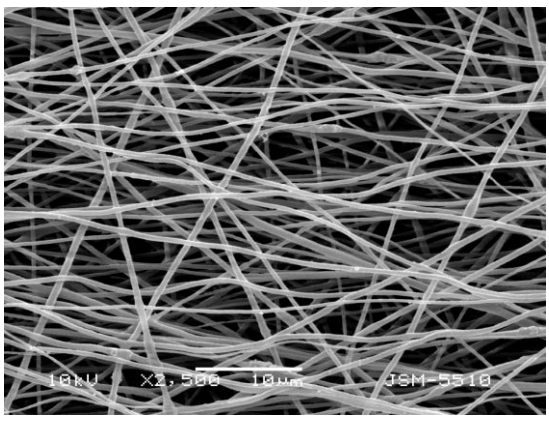

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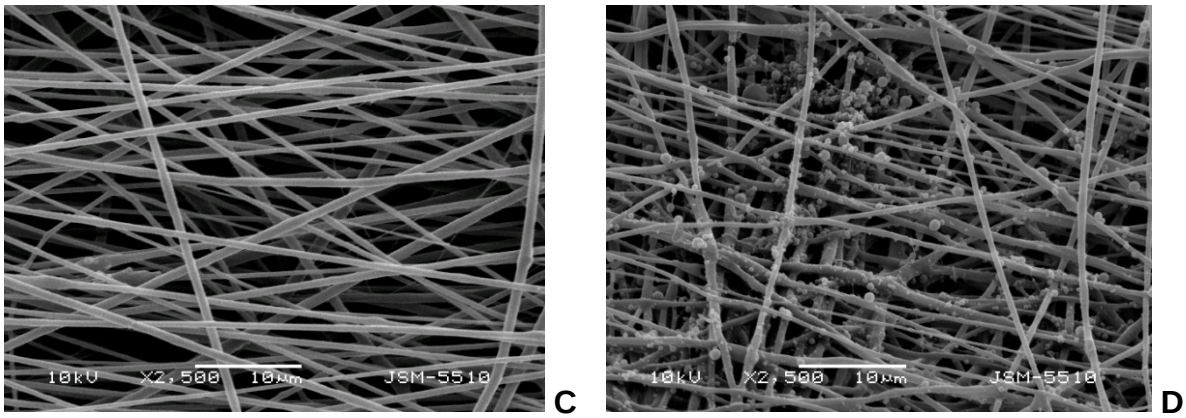


Figure C1-3. SEM micrographs of the fibers of: A. PVP*in*PHB, B. PVP,CQ*in*PHB, C. PHB, and D. PVP,CQonPHB. Magnification ×2500.

1.2. FTIR spectroscopy of fibrous materials

For confirmation of the chemical structure of the CQ-containing fibrous material the FTIR spectroscopy was carried out. In the FTIR spectra of PVP*in*PHB mats, in addition to the absorption characteristic bands of PHB (1721 cm^{-1} - νC=O, 1279, 1229, 1180 cm-1 - ν_{as} C-O-C in the crystalline and amorphous phases), a band appeared at 1663 cm^{-1}, characteristic for νC=O vibrations from the PVP. In the case of PVP,CQ*in*PHB mat the band for C=O stretching vibrations of PVP was shifted towards the higher wavenumbers to 1668 cm^{-1} (by 5 cm^{-1}) compared to the spectrum of the neat PVP*in*PHB mat (1663 cm^{-1}). The appearance of new bands for C=C stretching vibrations of CQ at 1576 cm^{-1} and 1489 cm^{-1}, as well as two bands at 808 cm^{-1} (for νAr-H, out-of-plane bending vibrations) and at 783 cm^{-1} (for aromatic C-H bonds) was also observed, which was an indication of the incorporation of CQ in the particles on the PHB mat surface.

1.3. Water contact angle measurements

The hydrophilic/hydrophobic characteristics of fibrous mats can greatly influence the adhesion and growth of pathogenic fungi in plants. It was of interest to measure the water contact angle of the obtained fibrous materials. We have found that the PHB mat was hydrophobic (water contact angle of 123.3° ± 1.6°) (Figure C1-5A). The water contact angle value of the PHB fibrous material determined experimentally by us was in good agreement with the reported in the literature values by other authors The incorporation of 10 wt% water-soluble polymer PVP resulted in a slight decrease in the hydrophobicity of the mats (the value of the water contact angle was 115.7° ± 1.7°,). The presence of CQ in the PVP,CQ*in*PHB fibers did not

result in a change in the water contact angle values (Figure C1-5C). Moreover, we have find out that the mats fabricated by electrospinning of PHB solution in conjunction with electrospraying of PVP,CQ were hydrophilic. Their water contact angles were 0° (Figure C1-5D).

Figure C1-5. Images of water droplets (10 µl) deposited on the surfaces of mats from A. PHB, B. PVP*in*PHB, C. PVP,CQ*in*PHB, and D. PVP,CQ*on*PHB. The direction of the collector rotation is demonstrated by an arrow.

1.4. Differential scanning calorimetry

The thermal behavior of the PVP,CQ*in*PHB and PVP,CQ*on*PHB mats was studied by DSC analyses (Figure C1-6). An endothermic peak at 170°C for T_m of PHB was detected in the thermogram of the PHB fibers (Figure C1-6B). Furthermore, in the thermograms of the PVP,CQ*in*PHB mats a 3 cm^{-1} shift of the T_m for PHB towards a lower temperature to 167°C was observed (Figure C1-6D). The crystallinity degree of PHB in PVP,CQ*in*PHB mats (37%) did not change significantly compared to that of PVP*in*PHB mats (42%). It can be assumed that when CQ is incorporated in the bulk of the PVP*in*PHB mat, interactions occur between PHB, PVP and CQ, resulting in diminishing of T_m of PHB. An endothermic peak at 170°C ascribed to PHB melting was registered in the thermograms of the mats obtained by electrospinning in conjunction with electrospraying (Figure C1-6 E). The crystallinity degree of PHB in PVP,CQ*on*PHB mats was 42%, a value that coincides with the crystallinity degree of the PHB fibers (42%). In the cases of PVP powder (Figure C1-6 A), and of fibrous mats containing PVP in the bulk of the mat or on its surface, a broad endothermic peak was detected between 25°C and 100°C, due to loss of moisture (Figure C1-6 C-E).

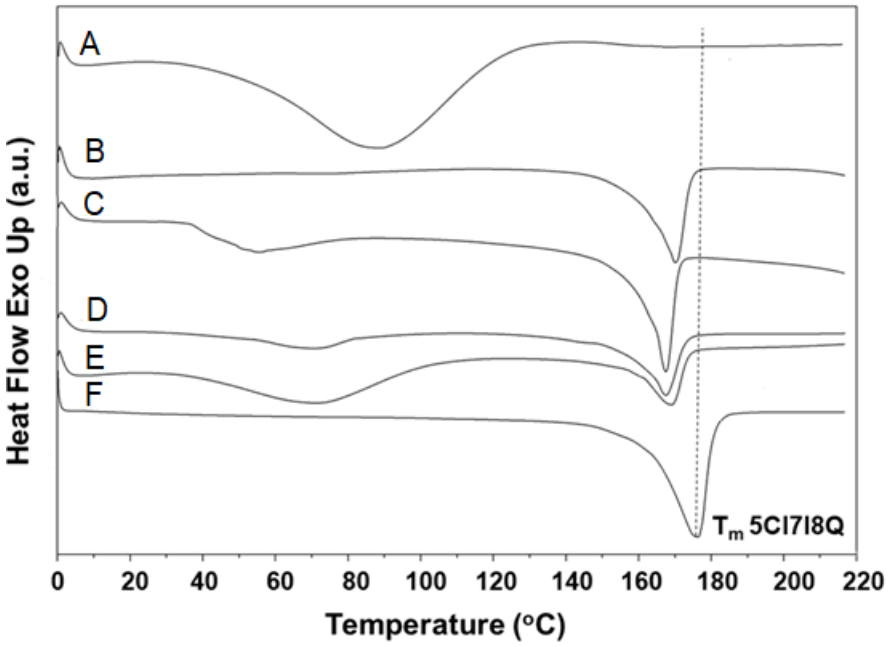


Figure C1-6. DSC thermograms (first heating run) of mats of: A. PVP powder, B. PHB mat, C. PVP*in*PHB mat, D. PVP,CQ*in*PHB mat, E. PVP,CQ*on*PHB mat and F. CQ powder.

Moreover, in the thermograms of the PVP,CQ*in*PHB mats no peak at 176°C corresponding to CQ melting was observed (Figure C1-6D). This demonstrated that CQ incorporated in the fibers was in the amorphous state. It can be seen that CQ incorporated in the particles deposited on the PHB fibers was also in the amorphous state (Figure C1-6E)

1.5. XRD Analysis

In XRD graph of the PHB and PVP*in*PHB fibrous materials only diffractions due to the crystalline phase of PHB (2θ = 13.6º, 17.0º, 20.1º, 22.1º, 25.6 º and 27.2 º were registered. In the cases of PVP,CQ*in*PHB and PVP,CQ*on*PHB mats (the main diffractions attributed to the crystalline phase of CQ were not observed (2θ = 6.3 º, 12.8 º, 20.4 º, 21.7 º and 24.6 º), thus demonstrating that CQ loaded into the mats or in the PVP particles on the PHB fibers was in the amorphous state. Thus obtained results were in conformity with the results obtained by DSC analyses.

1.6. Determination of chemical surface composition

The successful incorporation of CQ into the PVP*in*PHB mat surface or into the PVP particles deposited on the PHB mat surface was also confirmed by XPS analyses. The appearance of N_{1s} peaks in the spectra of the PVP,CQ*in*PHB mats

was observed at 399.2 eV due to –N-C=O from PVP and at 400.0 eV characteristic of –N-C from CQ (Figure C1-8C). Moreover, the spectra displayed the appearance of an I_{3d} peak - at 620.7 eV ($I_{3d5/2}$) and at 632.2 eV ($I_{3d3/2}$), attributed to the presence of CQ in the mat surface. Cl_{2p} (at 201.8 eV ($Cl_{2p1/2}$) and at 200.2 eV ($Cl_{2p3/2}$) peaks were also detected confirming the incorporation of CQ in the PVP,CQ*in*PHB mat surface. Five peaks were detected in the detailed C_{1s} spectrum of the PVP,CQ*in*PHB mat. The signal at 285 eV was ascribed to -C-H or -C-C- from PHB, PVP and from CQ, and that at 286.5 eV was attributed to -C-O-C, -C-OH from PHB, to C-N-C=O from PVP and also to -C-N and –C-OH from CQ. The peak at 287.4 eV was corresponding to -N-C=O from PVP and at 288.9 eV to -O-C=O from PHB. The presence of a peak at 290.4 eV for the $\pi \rightarrow \pi^*$ shake-up satellite due to the aromatic ring of the incorporated CQ was registered. Four components demonstrated the detailed O1s spectrum - at 531.5 eV ascribed to -N-C=O from PVP, at 532.0 eV to -C=O from PHB, at 532.5 eV to -C-OH from CQ and at 533.2 to -C-OH and -C-O from PHB. The presence of the detected peaks are in accordance with the structure of the PVP,CQ*in*PHB fibrous material. The theoretical ratio of the peak area for the respective carbon atoms was [C–C/C–H]/[C–O/C–OH/C-N-C=O/C-N/C-OH]/[N-C=O]/[O–C=O][$\pi \rightarrow \pi^*$] = 50.3/27.2/1.3/20.2/1.0, while the experimental ratio was 50.6/27.4/1.2/20.2/0.6. Therefore, the largest area was registered for the peak of the carbon atoms engaged in the C–C/C–H bonds. The obtained results were in conformity with the hydrophobicity of the surface of the PVP,CQ*in*PHB fibrous material.

Significant differences in the expanded C_{1s} spectrum of the PVP,CQ*on*PHB mat were registered in comparison to the C_{1s} spectrum of the PHB mat. Two new peaks at 287.3 eV ascribed to –N–C=O from PVP and at 290.4 eV to the $\pi \rightarrow \pi^*$ shake-up satellite of the CQ aromatic ring were detected. There was also an increase in the intensity of the peak at 286.4 eV ascribed to –C–O–C and –C–OH from PHB, to –C–N–C=O from PVP, as well as to -C-N and –C-OH from CQ. In the detailed O_{1s} spectrum of PVP,CQ*on*PHB fibrous material, the presence of two new peaks was identified - at 531.6 eV, corresponding to –N-C=O from PVP and at 532.6 eV, ascribed to –C–OH from CQ. The comparison of the detailed N_{1s} spectrum of these fibrous materials with that of the PHB mats revealed the presence of two new components - at 399.1 eV characteristic for –N-C=O from PVP and at 400.0 eV due

to –N-C from CQ. The presence of peaks for N_{1s}, I_{3d} (at 620.7 eV ($I_{3d5/2}$) and at 632.2 eV ($I_{3d3/2}$)) and Cl_{2p} (at 201.6 eV ($Cl_{2p1/2}$) and at 200.0 eV ($Cl_{2p3/2}$)) confirmed the incorporation of CQ into PVP particles deposited on the surface of PHB mat.

1.7. . In Vitro CQ Release Studies

The *in vitro* study of the CQ release from PVP,CQ*in*PHB and PVP,CQ*on*PHB mats was assessed spectrophotometrically in acetate buffer (pH 3.6) containing Tween 80 (99/1 v/v), for 48 h at 25°C. These fibrous materials containing the water-soluble polymer PVP showed rapid initial release with subsequent gradual release profile (Figure C1-11). As seen from Figure C1-11, CQ was released faster and in a greater amount when incorporated into PVP particles that are deposited on the PHB fibers` surface than when incorporated in the PVP*in*PHB fibers. About 78.6% and 64% of the loaded CQ was released in the initial 840 min in the case of PVP,CQ*on*PHB and PVP,CQ*in*PHB mats, respectively (Figure C1-11).

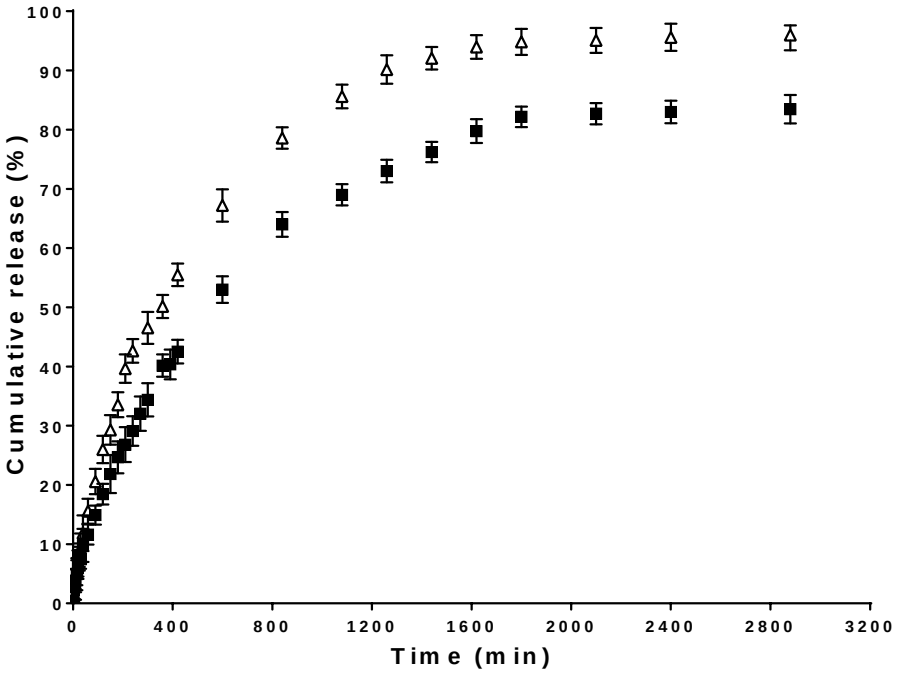


Figure C1-11. In vitro study of the CQ release from: PVP,CQ*in*PHB mat (■) and PVP,CQ*on*PHB mat (Δ) acetate buffer/Tween 80 (99/1 v/v) at 25°C, pH 3.6, ionic strength 0.1. Results are presented as mean values from three separate measurements with the corresponding standard deviation.

The amount of CQ released from the PVP,CQ*in*PHB mats for 2880 min was ca. 83.5%. For PVP,CQ*on*PHB fibrous materials the total amount of CQ released in 2880 min was 96%. This result might be due to the difference in the diffusion of CQ

incorporated in the bulk of the fibers and the diffusion of CQ through the PVP particles deposited on the fiber surface. The obtained results from the CQ release studies showed that the CQ release from the fibrous materials was assisted by the presence of PVP in the fibrous materials or on their surface. These results are consistent with our previous findings on an increase in the rate of release of 8-hydroxyquinoline derivatives from other fibrous systems upon incorporation of a water-soluble polymer.

1.8. Investigation of the antifungal activity of fibrous materials

8-hydroxyquinoline derivatives are known for their good antibacterial and antifungal properties. Among the 8-hydroxyquinoline derivatives, CQ manifested the ability to inhibit the growth of a large number of fungi like *C. albicans* etc. Until now, no data on the antifungal activity of CQ, as well as of fibrous materials containing this biologically active compound against *P. chlamydospora* and *P. aleophilum*, which are the main fungal species causing esca disease, have been reported.

Therefore, we have studied, the antifungal activity of fibrous materials loaded with CQ by performing microbiological assays against the fungi *P. chlamydospora* and *P. aleophilum*. The diameters of the inhibition zone around the fibrous discs and MIC values for CQ against the two used fungi were determined as well. The MIC values were 10 and 1 μg/ml, respectively. The growth of *P. chlamydospora* and *P. aleophilum* was studied for the time period of 96 h. The PHB and PVP*in*PHB mats did not show any significant antifungal effect. In contrast, CQ-containing mats exhibited antifungal activity against these fungi and well-defined zones of inhibition of fungal cell growth were detected (Figure C1-12B,D,F,H). These well-defined zones illustrated that the release profile of CQ provided a sufficient amount of the biologically active compound even in the initial experiment stages. The values of the mean diameter of the inhibition zones for PVP,CQ*in*PHB and PVP,CQ*on*PHB mats for the tests against *P. chlamydospora* did not differ significantly: 44.2 ± 1.1 mm and 45.0 ± 1.3 mm, respectively (Figure C1-12 B,D).. For the tests against *P. aleophilum*, the diameters of the zones of inhibition around PVP,CQinPHB and PVP,CQonPHB fibrous materials were 36.7 ± 1.9 and 41.2 ± 3.0 cm, respectively (Figure C1-12 F,H). The obtained results indicated that the incorporated CQ imparted good antifungal activity against these fungi. However, up to now the mechanism of action of CQ in

fungal cells has not been fully clarified. There are no data in the literature on the mechanism of action of CQ against fungal cells of *P. chlamydospora* and *P. aleophilum*. We hypothesize that the observed antifungal activity of CQ-containing fibrous materials against the fungi *P. chlamydospora* and *P. aleophilum* is most likely due to their damaging effect on the fungal cell wall.

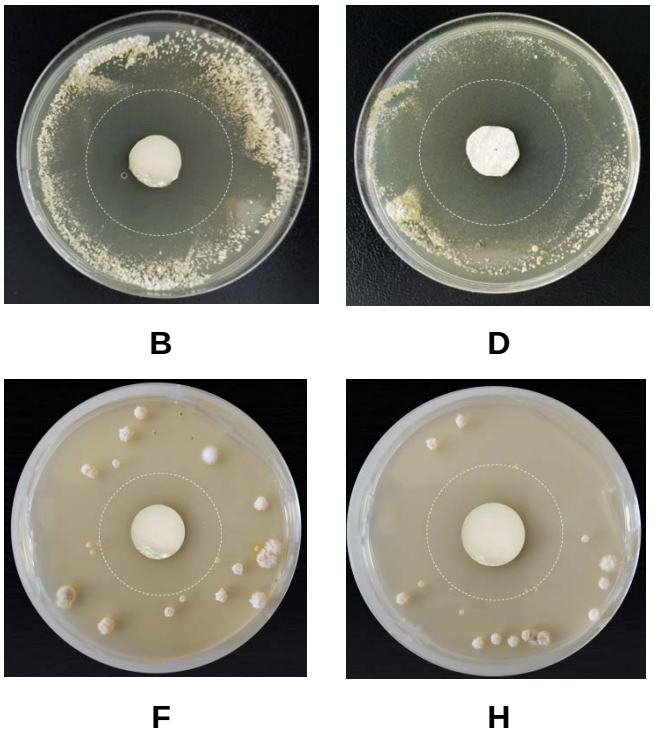
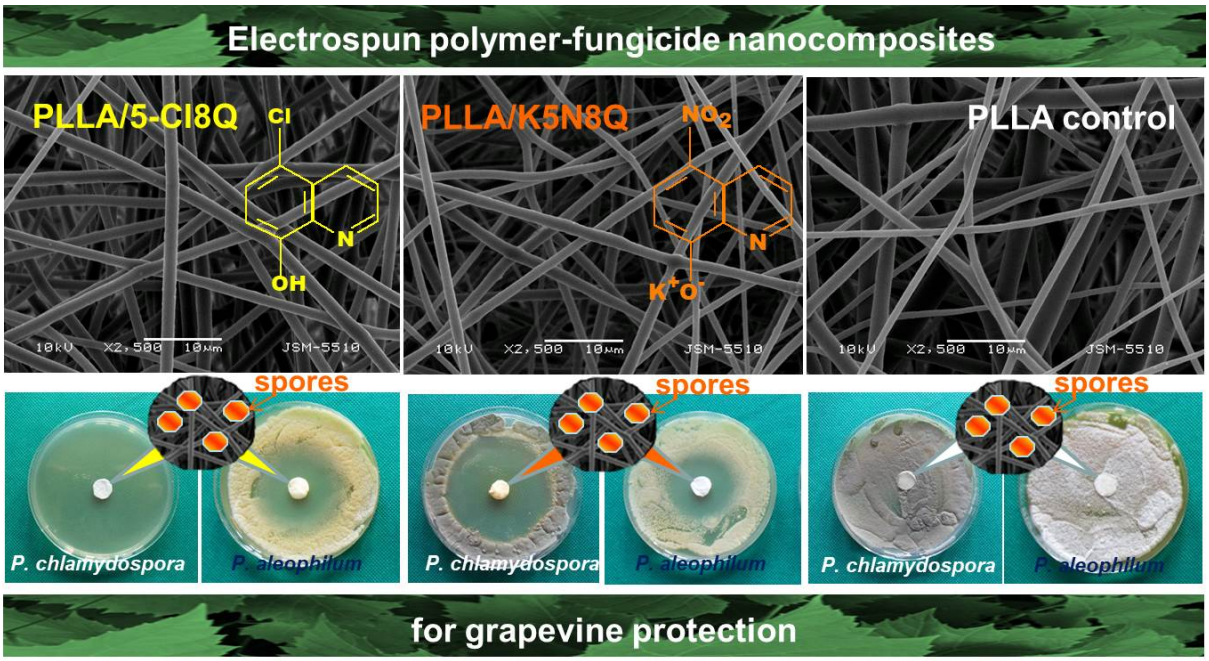


Figure C1-12. Digital images of the zones of inhibition against *P. chlamydospora* (B and D) and *P. aleophilum* (F and H) after the 96-hours contact of the fibrous materials with: B and F- PVP,CQ*in*PHB mat and D and H- PVP,CQ*on*PHB mat.

The obtained results revealed that CQ -containing fibrous materials with different designs can be obtained by electrospinning ("*in*" strategy) or by electrospinning in conjunction with electrospraying ("*on*" strategy). We found that the CQ incorporated into the bulk of the PVP*in*PHB fibers or the PVP particles deposited on the PHB fibers was in an amorphous. Furthermore, CQ-containing fibrous materials (both "in" and "on" types) exhibited significant antifungal activity. All these results clearly reveal that the prepared fibrous materials are promising as active dressings for protection of grapevine from growth and penetration of two main esca-causing fungal pathogens.

Chapter 2. ELECTROSPUN MICROFIBROUS PLLA-BASED MATERIALS WITH ANTIFUNGALL PROPERTIES. ACTIVE PROTECTION AGAINST *P. CHLAMYDOSPORA* AND *P. ALEOPHILUM* [P 2] [OP 4]



This Chapter summarizes the results on the preparation and characterization of novel microfibrous materials from PLLA and BAS: 5-Cl8Q and K5N8Q included (Figure C2-1) by the electrospinning. The morphology of the fibrous materials was evaluated by scanning electron microscopy. The resulting materials are hydrophobic and have good physical and mechanical properties. *In vitro* studies demonstrated that the fungicide release was higher from PLLA/K5N8Q fibrous mats compared to the released drug amount from PLLA/5-Cl8Q materials which is due to the better water solubility of potassium 5-nitro-8 -quinolinolate.

Figure C2-1. Structure of: A. 5-chloro-8-hydroxyquinoline (5-Cl8Q) and B. potassium 5-nitro-8-quinolinolate (K5N8Q).

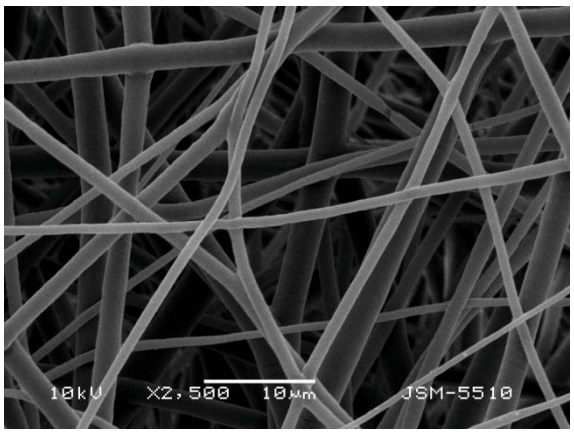
The antifungal activity of the fibrous materials against *P. chlamydospora* and *P. aleophilum* was studied as well. The incorporation of the fungicide in the

biodegradable fibers resulted in the inhibition of fungal growth. The obtained materials are perspective candidates for the protection of vines from the penetration and growth of fungal pathogens.

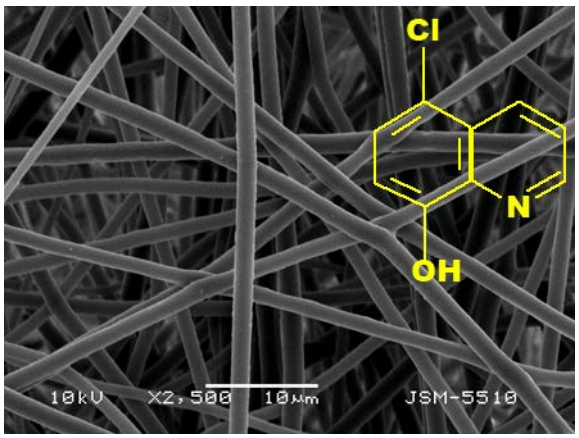
2.1. Preparation and morphology of PLLA-based fibrous materials incorporating 8-hydroxyquinoline derivatives

PLA is a biodegradable, biocompatible and thermoplastic polymer obtained from annually renewable resources. In addition, this polymer has excellent physico-mechanical characteristics. Due to its merrits, PLA is a particularly suitable carrier for agropharmaceuticals (APS).

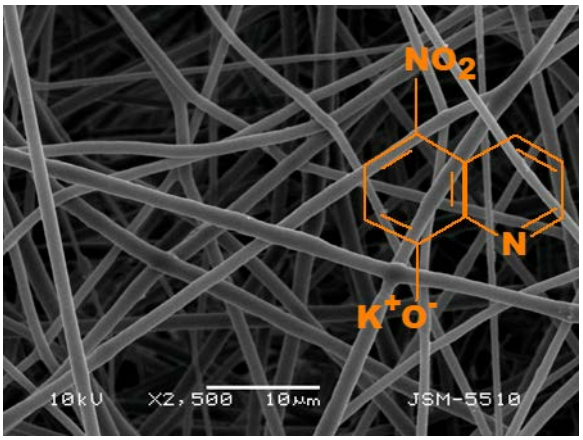
In previous studies carried out in LBAP, it was found that some 8-hydroxyquinoline derivatives inhibited the growth of *P. chlamydospora* and *P. aleophilum*. In order to investigate the effect of the solubility of some derivatives, we used: 5-chloro-8-hydroxyquinoline (5-Cl8Q) and potassium 5-nitro-8-quinolinolate (K5N8Q). Figure C2-2 presents SEM micrographs of fibers obtained by electrospinning of a spinning solution of PLLA (concentration 10 wt%) and 5-Cl8Q and K5N8Q (at a concentration of 10 wt% based on polymer weight) in mixed DCM /EtOH solvent system (DCM/EtOH = 90/10). The mean values of PLLA fiber diameters were 1045 ± 320 nm (Figure C2-2 A). The obtained diameters of the PLA fibers are in fairly good agreement with the literature data.



A 1045 ± 320 nm



B 1125 ± 300 nm



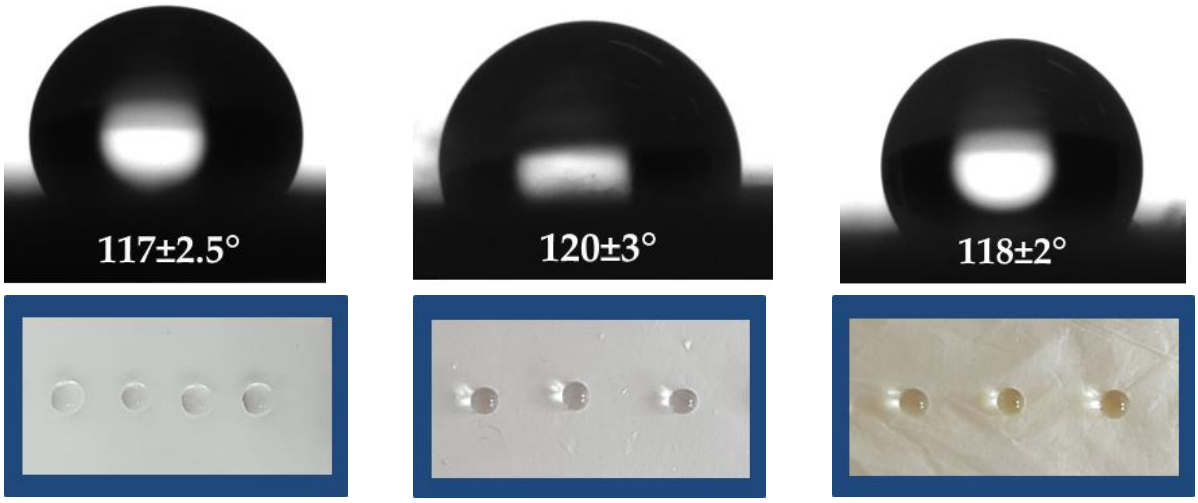
C 1065 ± 250 nm

Figure C2-2. Representative SEM images of the fibers of electrospun fibrous materials of A. PLLA, B. PLLA/5-Cl8Q and C. PLLA/K5N8Q; magnification ×2500.

As can be easily seen uniform fibers with a cylindrical shape were obtained. The average diameter of the fibers of the fibrous materials based on PLLA/5-Cl8Q and PLLA/K5N8Q was 1125 ± 300 nm and 1065 ± 250 nm, respectively. This is an indication that the addition of low molecular fungicides (derivatives of 8-hydroxyquinoline) did not lead to a significant change in the fiber morphology or diameters and distribution.

2.2. *Water contact angle measurements*

The values of the water contact angles for all obtained samples were determined. The PLLA fibrous material was hydrophobic, with a water contact angle of 117° ± 2.5° (Figure C2-3 A). The measured value for the pure PLLA was close to the values found in the literature.



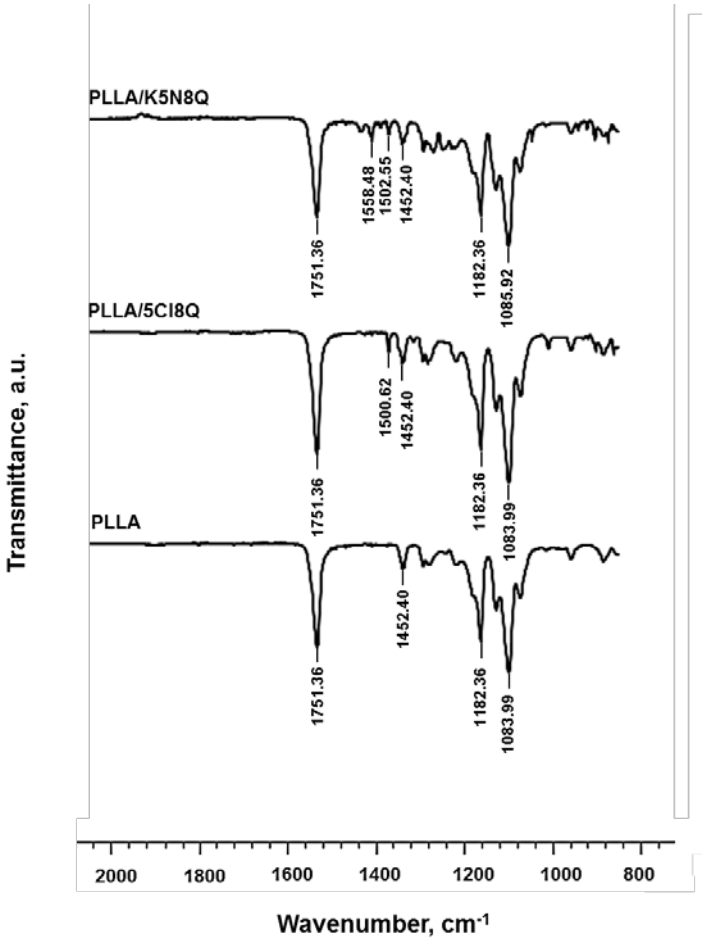
A **B** **C**

Figure C2-3. Images of water droplets deposited on the surface of fibrous materials: A. PLLA, B. PLLA/5-Cl8Q, and C. PLLA/K5N8Q.

The measured contact angle values of PLLA/5-Cl8Q and PLLA/K5N8Q composite fibrous materials were 120° ± 3° and 118.0°◦ ± 2°, respectively (Figure C2-3 B,C). The measured water contact angle values were close to those measured for the PLLA electrospun material.

2.3. FTIR spectroscopic analysis

The composition of the obtained fibrous materials was qualitatively characterized by FTIR spectroscopy. The recorded spectra are shown in Figure C2-4.



Фигура C2-4. FTIR spectra of electrospun fibrous materials of: PLLA, PLLA/5-Cl8Q and PLLA/K5N8Q.

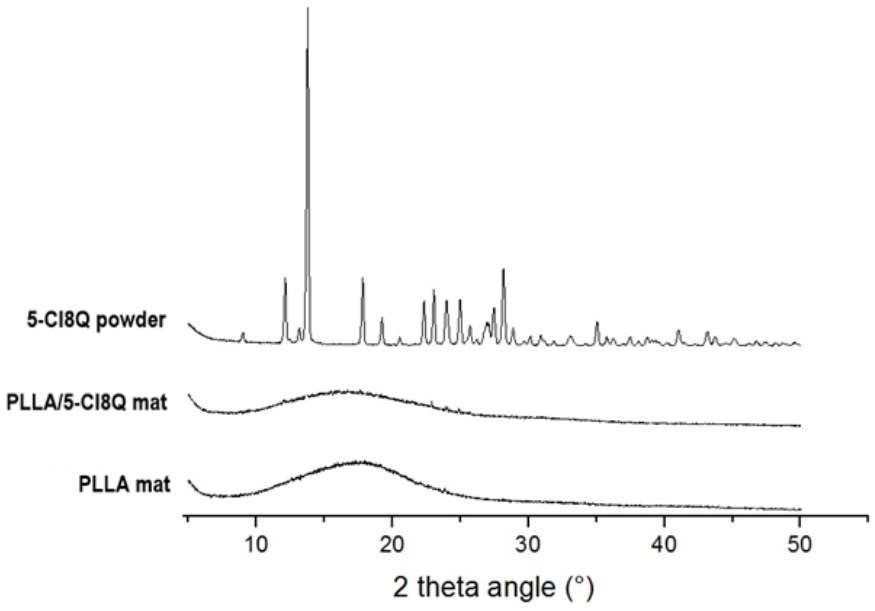
In the IR spectrum of PLLA material bands characteristic for PLLA appeared at 1751 cm^{-1} for the C=O groups and at 1182 cm^{-1} for C–O–C groups. Characteristic stretching frequencies for C–O at 1080 cm^{-1} and bending frequencies for $–CH_3$

asymmetric and $–CH_3$ symmetric have been identified at 1452 cm^{-1} and 1361 cm^{-1}, as well in accordance with literature.

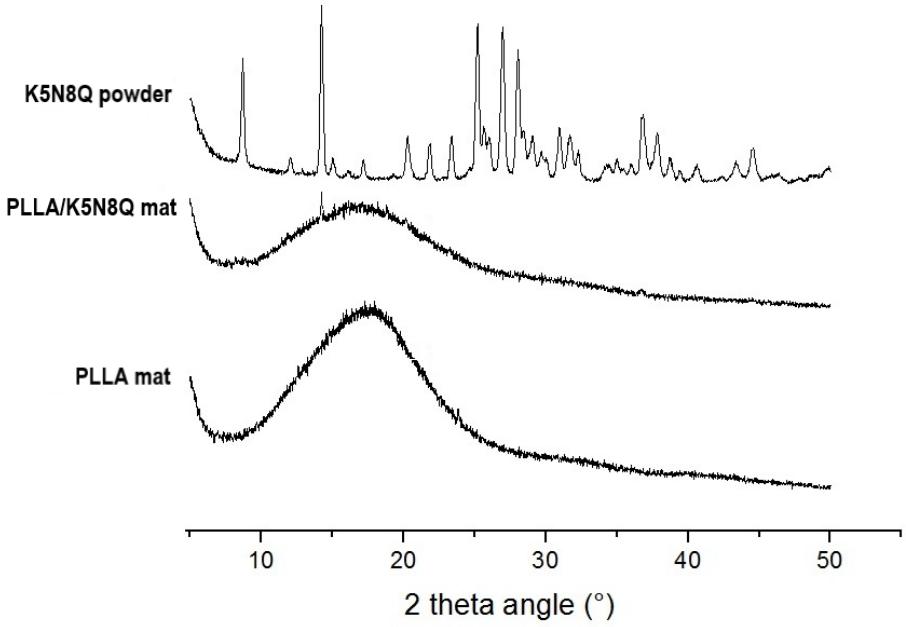
In the FTIR spectra of composite PLLA/5-Cl8Q and PLLA/K5N8Q fibrous materials in addition to the characteristic bands of PLLA new band appeared at 1500 cm^{-1} characteristic for aromatic ring of the chemical fungicide, proving the presence of it in the electrospun composite materials.

2.4. XRD analysis

The crystallinity of the obtained fibrous materials was determined by X-ray diffraction (XRD) analysis (Figure C2-5). XRD pattern of PLLA and PLLA/5-Cl8Q mats and 5-Cl8Q powder, as well as of PLLA/K5N8Q fibrous mat and K5N8Q powder are shown in Figure C2-5 A and C2-5 B. As it could be easily seen in the XRD patterns of 5-Cl8Q and K5N8Q (powder) characteristic sharp diffraction peaks of the compounds were observed. These peaks showed that the fungicides (powders) were highly crystalline. XRD spectra of PLLA fibers showed a strong amorphous halo proving that these materials have a typical amorphous structure. Moreover, in the spectra of PLLA/5-Cl8Q and PLLA/K5N8Q composite materials amorphous halo was detected as well. This result indicates that each component in the composite fibrous materials prepared by electrospinning was in an amorphous state.



A



B

Figure C2-5. X-ray diffraction pattern of: A. 5-Cl8Q powder, PLLA and PLLA/5-Cl8Q mat and B. K5N8Q powder, PLLA and PLLA/K5N8Q mat.

2.5. Mechanical properties

Typical stress-strain curves of PLLA, PLLA/5-Cl8Q and PLLA/K5N8Q electrospun fibrous materials are shown in Figure C2-6. The PLLA mat showed the highest values of tensile strength. The determination of the mechanical characteristics of the PLLA/5-Cl8Q and PLLA/K5N8Q composite fibers shows that these materials manifest mechanical properties similar, however, a bit lower than those of PLLA fibrous mats. This result indicates that the incorporation of the 5-Cl8Q or K5N8Q in the fibrous membranes does not considerably alter the mechanical characteristics of the obtained materials. The tensile strength of the PLLA/5-Cl8Q and PLLA/K5N8Q composite fibrous mats was ca. 2.5 MPa while the tensile strength of the PLLA fibrous materials reaches 3.4 MPa. The slight decrease in mechanical characteristics might be attributed to the incorporation of the low-molecular weight chemical fungicides in the PLA matrix that might generate weakness spots when the tensile test was carried out.

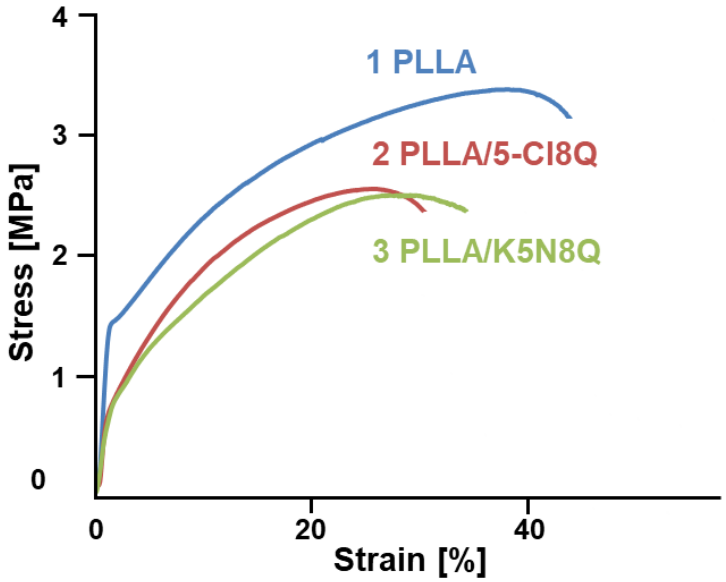


Figure C2-6. Stress–strain curves of electrospun materials: (1) PLLA, (2) PLLA/5-Cl8Q and (3) PLLA/K5N8Q.

2.6. Cumulative drug release analysis

The release profile of 5-Cl8Q and K5N8Q from PLLA fiber mat was studied spectrophotometrically using acetate buffer (CH_3COONa/CH_3COOH), containing lactic acid (acetate buffer/lactic acid = 96/4 v/v) at pH 3 and ionic strength 0.1, at 37°C. The release profile is shown in Figure C2-7. Initially, the both drugs showed a relatively burst release from the PLLA fibrous matrix. However, K5N8Q was released in higher amount compared to 5-Cl8Q for the same duration. For instance, the released K5N8Q was 10.5 and 21.4% for 30 and 60 min. For the same time durations the released 5-Cl8Q was 6.7% and 9.3%, respectively. This difference in the released profile could be attributed to the different nature of the drugs and their water-solubility. The K5N8Q is a partially water soluble drug favoring more rapid release. On the other hand 5-Cl8Q is water insoluble which hampers its release. After 50 h, the amounts of the released 5-Cl8Q and K5N8Q were 52.8% and 72.5%, respectively.

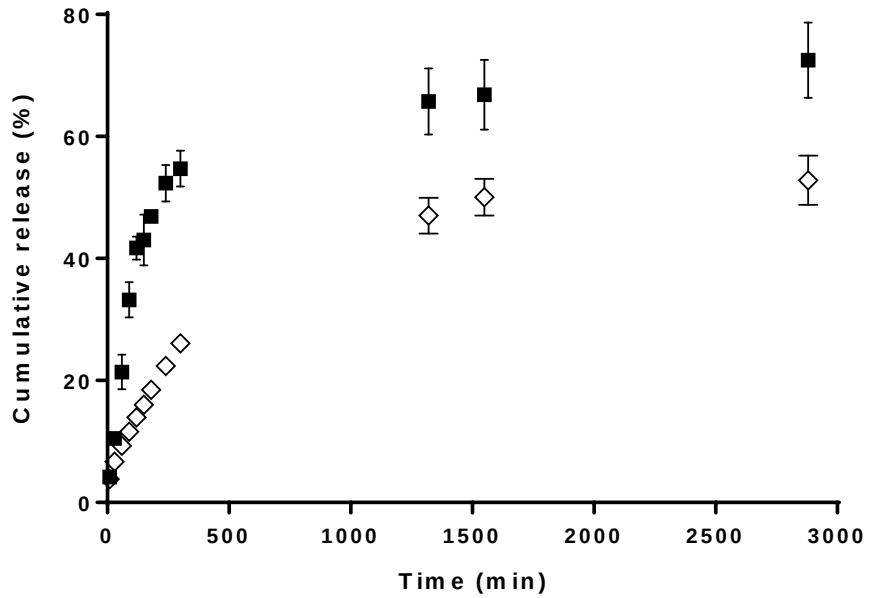


Figure C2-7. Release profiles of 5-Cl8Q and K5N8Q from PLLA fibers: PLLA/5-Cl8Q (◊) and PLLA/K5N8Q (■). The results are presented as average values from three separate measurements with the respective standard deviation; acetate buffer/lactic acid (96/4 v/v), pH 3, 37°C, ionic strength 0.1.

2.7. Antifungal activity of the fibrous membranes against P. chlamydospora and P. aleophilum

The antifungal activity of the electrospun PLLA, PLLA/5-Cl8Q and PLLA/K5N8Q fibrous materials was assessed by performing antifungal tests against *P. chlamydospora* and *P. aleophilum*. Although there are data regarding the antifungal activity of 8-hydroxyquinoline derivatives against fungi of the Candida species, there are no data on their effects against the fungi *P. chlamydospora* and *P. aleophilum*, which are the main causative agents of the esca disease. For this reason, we initially determined the minimum inhibitory concentration (MIC) of 5-Cl8Q against both fungal species and it was 0.75 μg/mL for both strains. For K5N8Q, the determined MIC against *P. chlamydospora* and *P. aleophilum* were 12.5 μg/mL and 25 μg/mL, respectively.

The results obtained by determination of the zones of inhibition after contact of the fibrous materials with the fungal cells are shown in Figure C2-8. The incorporation of 5-Cl8Q in the composite fibrous materials that were placed in contact with *P. chlamydospora* resulted in complete inhibition of the fungal growth. Moreover, there is wide inhibition zone around the PLLA/5-Cl8Q disc placed in contact with *P.*

aleophilum (4.7 cm). Additionally, the incorporation of the K5N8Q resulted in observation of wide zones of inhibition as well. The diameters of the inhibition zones around the PLLA/K5N8Q discs are 6.2 cm and 4.0 cm against *P. chlamydospora* and *P. aleophilum*, respectively. From the obtained results it is easily seen that *P. chlamydospora* is more vulnerable to treatment with the used 8-hydroxyquinoline derivatives.

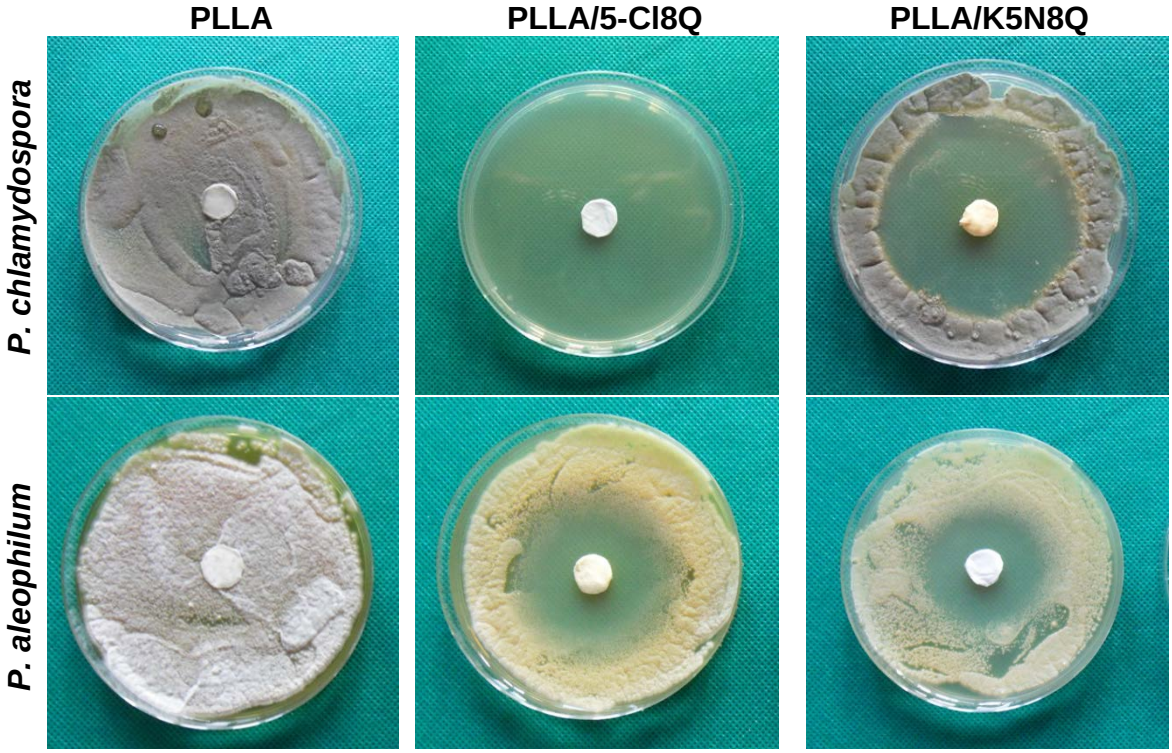
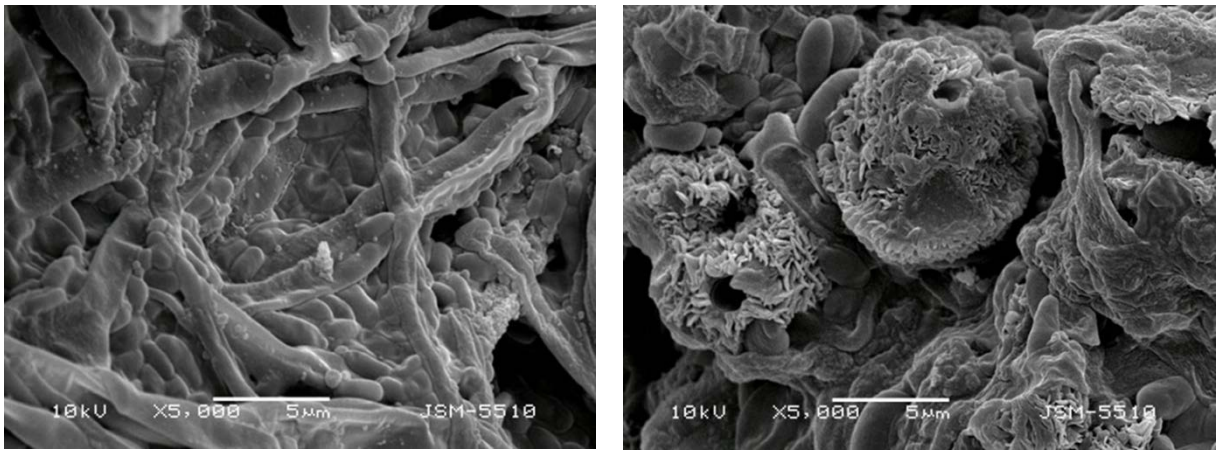


Figure C2-8. Digital images of the zones of inhibition against *P. chlamydospora* and *P. aleophilum* after contact of the fibrous materials with fungi cells. The material type is indicated at the top of each column. The cell type is marked in the left of each row.

The obtained results demonstrate that the composite fibrous materials containing hydroxyquinoline derivatives exhibited strong antifungal activity. In contrast, the neat PLLA fibrous materials did not alter the fungal growth and did not exhibit any antifungal activity.

The barrier efficacy of PLLA, PLLA/5-Cl8Q and PLLA/K5N8Q electrospun fibrous materials was studied as well. For that purpose, 20 mL conidia suspension was passed through each fibrous material (diameter 45 mm) by using a filtration device. By using SEM analysis the size of the *P. chlamydospora* and *P. aleophilum* conidia in the fungal suspension has been determined. SEM micrographs of the conidia used in the present study are shown in Figure C2-9.

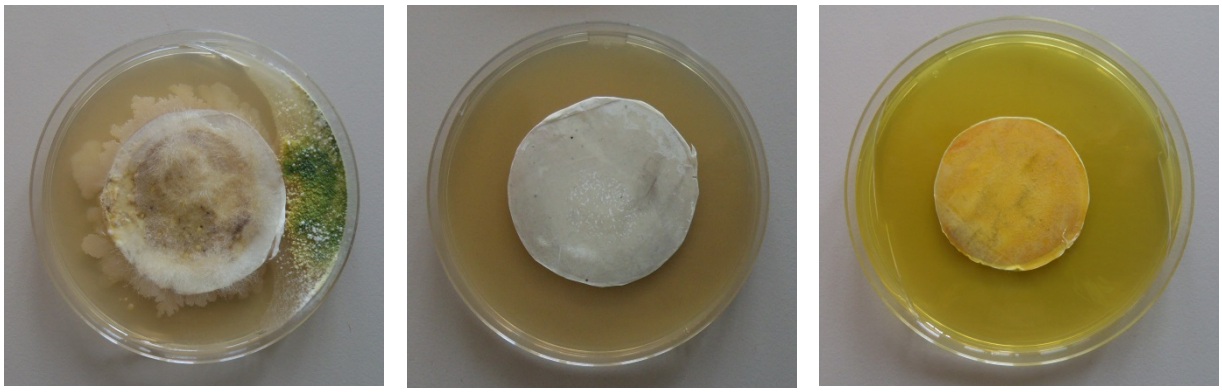


A **B**

Figure C2-9. SEM micrograph of the A. P. *chlamydospora* and B. *P. aleophilum* conidia.

The diameter and length of the *P. chlamydospora* conidia were ~0.75–1.2 μm and 1.8–2.3 μm, respectively. The measured diameter and length of the *P. aleophilum* conidia were ~1.1–1.5 μm and 2.5–3.5 μm. The initial concentration in the filtration experiments was 1×10^7 conidia/mL for the both strains. After passing through the electrospun fibrous discs the determined spore concentration was 1.6×10^3, 1.3×10^3 and 1.4×10^3 for the PLLA, PLLA/5-Cl8Q and PLLA/K5N8Q materials, respectively. This result revealed that the final conidia concentration decreased significantly. However, some conidia passed through all the fibrous mats obtained in this study.

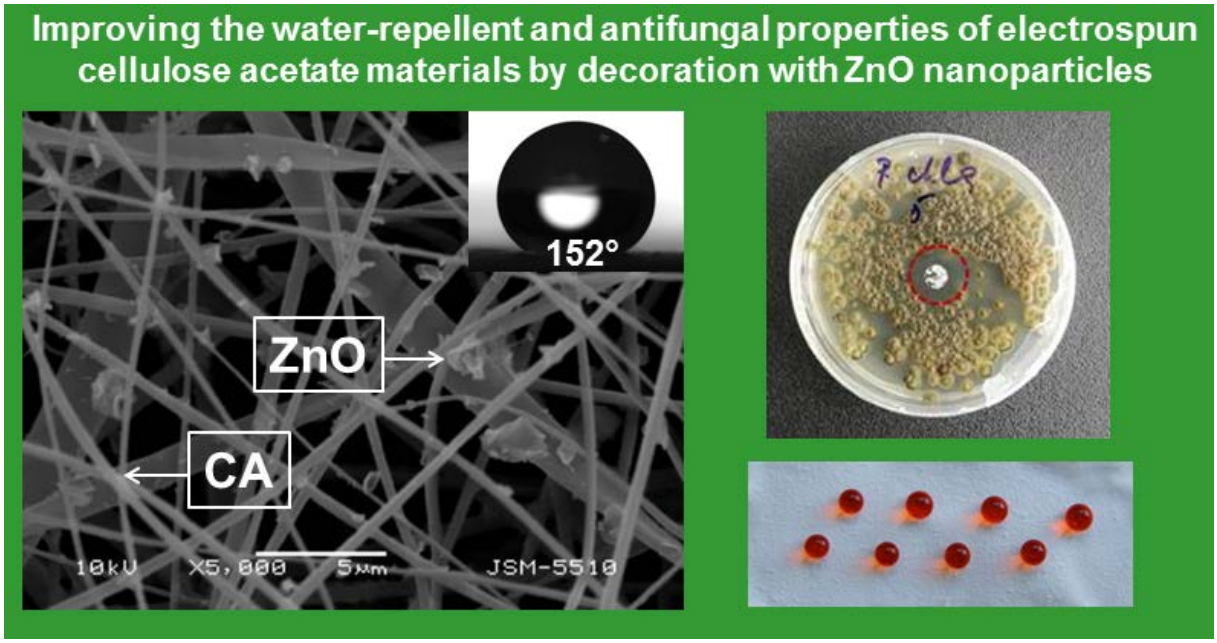
Therefore, it was of interest to study not only the barrier properties of the mats but also their antifungal activity after the conidia passing through them. After the filtration, we have placed the used disks on a surface of a solid agar in a Petri dish in order to determine the growth of the remaining in the fibrous discs fungi. The Petri dishes were incubated for 96 h at 28°C and then the fungal growth was assessed. The digital images of growth of *P. chlamydospora* on the fibrous materials surface were shown in Figure C2-10. It was found that the PLLA fibrous material used in filtration experiments developed colonies of *P. chlamydospora* (Figure C2-10 A). Colonies developed are proof that this material does not possess antifungal activity. The electrospun PLLA/5-Cl8Q and PLLA/K5N8Q materials, which were placed in appropriate conditions for the development of the remaining spores in the materials, impede the fungal growth resulting in compete fungal inhibition (Figure C2-10 B and C2-10 C). This result indicated that the fungi remaining in the PLLA/5-Cl8Q and PLLA/K5N8Q mats after the filtration through them could not grow due to the antifungal activity of the obtained composite materials.



A B C

Figure C2-10. Digital images of the growth *P. chlamydospora* on the fibrous materials: A. PLLA, B. PLLA/5-Cl8Q and C. PLLA/K5N8Q, used after spore filtration.

Chapter 3. ELECTROSPUN CELLULOSE ACETATE MATERIALS DECORATED WITH ZnO NANOPARTICLES WITH IMPROVED WATER-REPELLENT AND ANTIFUNGAL PROPERTIES[P 1] [PP 1,2] [OP 1,2]



In Chapter 1 and Chapter 2 we looked at the preparation of new innovative materials with fungicidal properties. They are obtained by the electrokinetic methods of electrospinning or electrospinning with simultaneous electrospraying. Three derivatives of 8-hydroxyquinoline were used – the monosubstituted 5-chloro- and 5-nitro-derivatives and the disubstituted 5-chloro-7-iodo derivative. To prepare the fibrous materials, we used the biodegradable polymers PHB and PLLA. It is of interest to investigate the possibility of obtaining mats with fungicidal activity from some cellulose derivatives and metal oxide (zinc oxide).

In **Chapter 3**, the possibility to obtain electrospun fibrous materials (defect-free fibers and fibers with defects) from cellulose acetate or cellulose acetate and ZnO is shown. A number of studies have been conducted to determine the optimum conditions for electrospraying or electrospinning of cellulose acetate in acetone/water. The effect of ZnO nanoparticles incorporation on morphology, wettability and antifungal activity against *P. chlamydospora* was evaluated by varying the concentration of CA. The obtained cellulose acetate fibrous materials decorated with nanoparticles could be suitable candidates to find potential applications in agriculture for crop protection.

3.1. Preparation and morphology of the fibrous materials from CA and ZnO

Cellulose acetate is one of the most important esters of cellulose. The advantages of CA are its low cost, ease of solubility in solvents suitable for electrospinning, facile production and wide variety of applications.

It was found, however, that none of the solvents - acetone, acetic acid and dimethylacetamide alone allowed continuous formation of cellulose acetate fibers. Cellulose acetate is insoluble in water, but when water is mixed with acetone and acetic acid, the vapor pressure of the system decreases, resulting in a significant improvement in the electrospinning process.

ZnO is nontoxic and exhibits photochemical and antibacterial activity. This is due to the fact that nanostructured ZnO is a very active material, since it can generate reactive oxygen species (ROS) and can release Zn^{2+} ions. Some of us have demonstrated the possibility to decorate fluorine-containing polymers with ZnO nanoparticles by applying electrospinning/electrospraying. We have shown that the obtained hybrid fibrous mats manifest antibacterial activity against the Gram-positive bacteria *Staphylococcus aureus*.

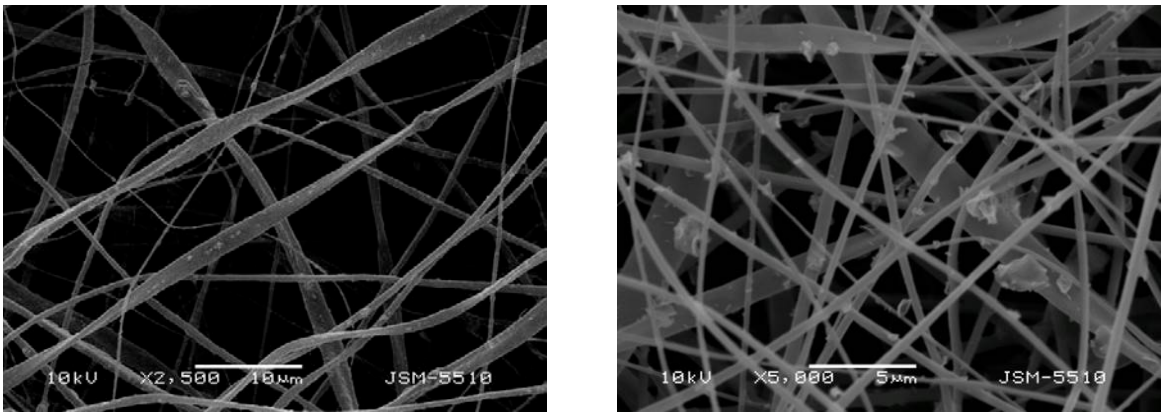
Solutions of cellulose acetate with different polymer concentration in mixed solvent acetone/water were prepared. The values of the dynamic viscosity of the prepared solutions were measured. The viscosity of the CA solutions in acetone/water with concentrations 6, 8, 10 and 17 wt% was 20 cP, 52 cP, 122 and 1120 cP, respectively. It was found that by increasing the polymer concentration the values of the solution viscosity increased. The increase in solution viscosity was attributed to the higher degree of chain entanglements.

The morphology of the prepared fibrous mats was observed by scanning electron microscopy. Electrospinning of cellulose acetate solution with concentration of 6 wt% and value of the solution viscosity of 20 cP resulted in preparation mostly of microparticles connected with fine fibers.

A transition from preparation of particles to formation of fibers with defects was observed when solution with concentration of 8 wt% and viscosity of 52 cP was subjected to electrospinning. The mean fiber diameters of the cellulose acetate fibers prepared from the solutions with concentrations 6 and 8 wt% were 680±119 nm and 780±110 nm, respectively. By increasing the concentration of cellulose acetate the number of defects decreased. It was found that the fibers prepared from solution of cellulose acetate with concentration of 10 wt% and values of the solution viscosity of 122 cP were uniform, defect-free and with ribbon-like structure. The preparation of ribbon-like fibers by electrospinning of cellulose acetate is consistent with the literature data. The formation of ribbons is explained by the formation of a thin polymer skin on the jet. After the skin formed, the solvent inside escaped. Atmospheric pressure tended to collapse the tube formed by the skin as the solvent evaporated. The circular cross section became elliptical and then flat, forming a ribbon.

By further increase of the concentration up to 17 wt%, however, the value of the solution viscosity increased to 1120 cP which led to obstruction of the flow from the capillary and instability of the process leading to formation of non-uniform fibers. The mean fiber diameter of the thin fibers was 580±180 nm and of the thicker fibers - 5.9±1.2 μm. It was found that the concentration of the cellulose acetate had a significant effect on the fiber morphology, while defect-free and uniform fibers were obtained by electrospinning of solutions of cellulose acetate with concentration of 10 wt%.

Representative SEM images of the obtained hybrid fibers of CA (10 wt%) and ZnO nanoparticles (30 wt%) with design type “in” (Figure C3-2 A) and “on” (Figure C3-2 B) are shown in Figure C3-2. The incorporation of ZnO (30 wt%) led to morphological changes in the obtained fibers and to increase of the mean fiber diameter. The increase in fiber diameter was attributed to the increase in the viscosity of the CA/ZnO suspension (2010 cP).



A **B**

Figure C3-2. SEM micrographs of electrospun hybrid materials of: A. ZnO-*in*-CA and B. ZnO-*on*-CA. Magnification x 5 000.

It is well known that the increase in molecular weight of the used polymer(s), polymer concentration and solution/suspension viscosity results in an increase of the mean fiber diameter. The mean fiber diameter of the hybrid ZnO-*in*-CA fibers was 1150±285 nm. It was observed that the incorporation of ZnO nanoparticles resulted in the preparation of fibers with rough surface in which zinc oxide was distributed mainly in the fibers' bulk; however some ZnO aggregates were formed. It may be assumed that one of the possible reasons for the presence of aggregates in the fibers is the solvent evaporation during the flight of the jet from the nozzle to the collector.

SEM micrographs of fibrous mats of ZnO-*on*-CA are presented in Figure C3-2 B. As seen the ZnO particles are distributed uniformly onto the fibers in the form of small and large particles. This results in decoration of CA fibers with ZnO particles.

3.2. Water contact angle of the mats

The water contact angle of the obtained CA fibrous mats was measured. It was found that the electrospun mats of CA were hydrophobic with water contact angle value 120º± 4˚.

The effect of ZnO particles on the wetting of cellulose acetate-based hybrid fibers was investigated. The images of the distilled water droplet (10 µl), deposited onto the surface of the ZnO-*in*-CA and ZnO-*on*-CA mats are shown in Figure C3-3 A and C3-3 B. The incorporation of ZnO particles with silanized surface resulted in the hydrophobization of the CA fibrous mats. The mean value of the water contact angle of ZnO-*in*-CA mat was 143.5±2.4° (Figure C3-3 A).

The preparation of novel fibrous hybrid materials based on CA and ZnO nanoparticles with design type "*on*" led to further increase of the water contact angle and to fabrication of materials with superhydrophobic properties with contact angles 152°±2° (Figure C3-3 B). For ease of visualization the water was coloured with reactive red, was dripped onto the mat and the drops were photographed (Figure C3-3 C). As it can be clearly seen the water droplets preserved their spherical and round shape and did not spread on to the surface.

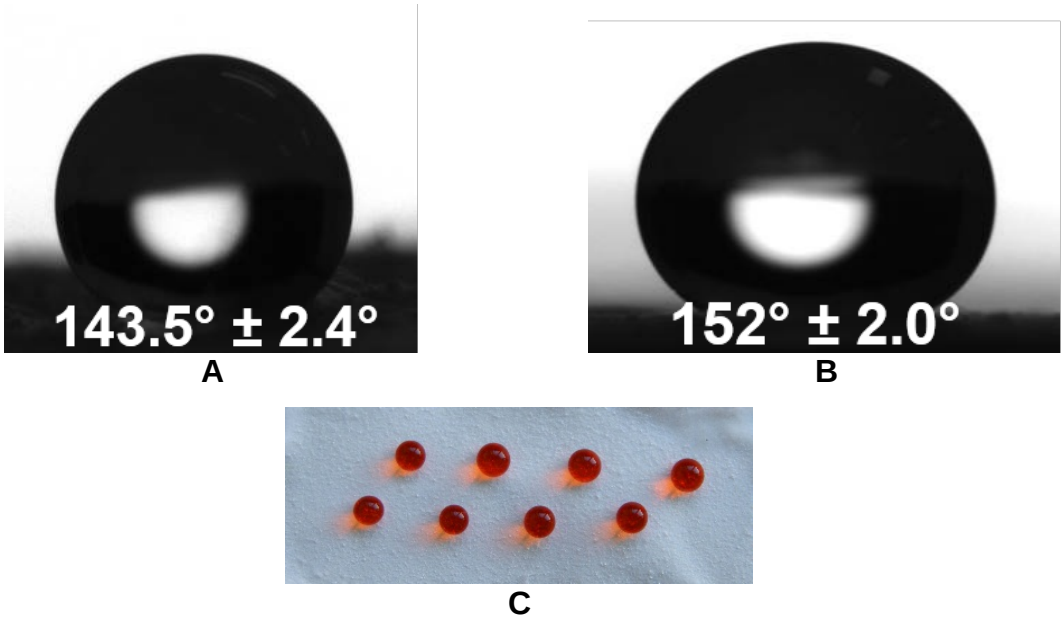


Figure C3-3. Digital images of distilled water droplet onto mats of: A. ZnO-*in*-CA and B. ZnO-*on*-CA. C. coloured with reactive red and deposited on the ZnO-*on*-CA mat**.**

Decorating CA fibers with ZnO particles led to the preparation of materials with uneven structure, large specific surface area, and superhydrophobic properties.

3.3. X-ray diffraction

XRD patterns of ZnO powder and CA and ZnO-on-CA mats recorded in 2θ range from 10 to 60° are presented in Figure C3-4.

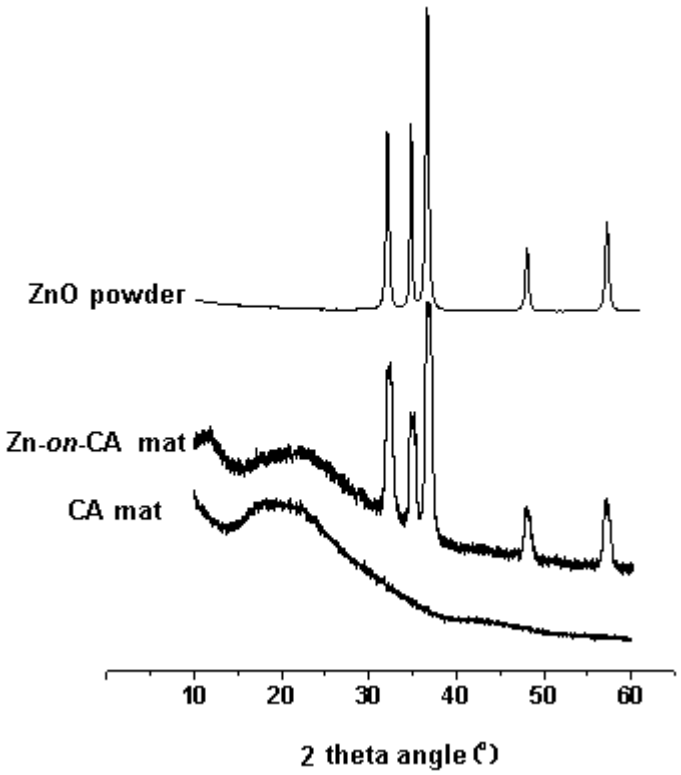


Figure C3-4. XRD patterns of ZnO powder, ZnO-*on*-CA mat and CA mat.

The presence of the main diffraction peaks of hexagonal wurtzite ZnO, (100) (002) (101) (102) and (110), located at 2θ=34.5°, 36.3°, 47.7° and 56.7° was found. Two broad peaks at 2θ = 10.3° and 21.7° were present in the XRD pattern of CA mat which reveals that the CA mat was in amorphous state. In the hybrid fibrous materials based on CA and ZnO materials, the XRD analysis revealed the presence of two broad peaks characteristic for CA and sharp peaks characteristic for ZnO. This confirms that all hybrid mats contained CA and ZnO as well.

3.4. Antifungal activity of the fibrous materials

The antifungal activity of the electrospun mats based on CA and CA/ZnO was assessed by performing tests against *P. chlamydospora*. The results obtained by the determination of the zones of inhibition after the contact of the fibrous materials with the fungal cells are shown in Figure C3-5.

A **B** **C**

Figure C3-5. Digital images of the zones of inhibition against *P. chlamydospora* after the contact of the fibrous materials with fungal cells: A. control, B. CA mat and C. ZnO-*on*-CA mat.

The fibrous CA mats did not alter the fungal growth and did not exhibit any antifungal activity (Figure C3-5 B). The ZnO-*in*-CA mat showed an insignificant inhibitory effect. The decoration of the surface of CA fibers with ZnO particles led to inhibition of the growth of the fungi and the formation of an inhibition zone (Figure C3-5 C). The diameter of the zone of inhibition was 16 mm. The observation of a zone of inhibition around the ZnO-*on*-CA mat is evidence that the ZnO deposited onto the fiber surface imparts antifungal activity to the prepared novel hybrid mats.

These properties indicate that fibrous materials based on cellulose and its derivatives decorated with ZnO could find application in agriculture for plant protection against adhesion and growth of pathogenic fungi.

IV. CONCLUSIONS

Novel eco-friendly electrospun polymer materials with antifungal activity have been prepared and possibilities for their application in agriculture have been shown.

The following conclusions can be drawn from the conducted research:

1. Fibrous materials containing CQ with diverse design were obtained by electrospinning (strategy type “*in*”) and by combining electrospinning and electrospraying (strategy type “*on*”).
2. CQ incorporated into of the PVP*in*PHB fibers or in the PVP particles deposited on the PHB fibers is in an amorphous state.
3. CQ-containing fibrous mats exhibit significant antifungal activity against the fungi *P. chlamydospora* and *P. aleophilum*.

4. Optimal conditions were found for the preparation of novel micro- and nanofibrous materials from PLLA and included biologically active substances: 5-Cl8Q and K5N8Q by the electrospinning method.

5. *In vitro* studies showed that the fungicide was released to a greater extent from the PLLA/K5N8Q fibrous mats compared to the amount of fungicide released from the PLLA/5-Cl8Q materials, which was due to the better water solubility of potassium 5-nitro -8-quinolinolate.

6. The obtained PLLA/5-Cl8Q and PLLA/K5N8Q fibrous materials were hydrophobic, with good physico-mechanical properties and possessed antifungal activity against *P. chlamydospora* and *P. aleophilum*.

7. Suitable conditions were found to prepare cellulose acetate (CA) or CA and ZnO nanoparticles fibrous materials by electrospinning and simultaneous electrospinning with electrospraying.

8. Surface decoration of CA fibers with ZnO nanoparticles resulted in fibers with superhydrophobic and antifungal properties.

9. The obtained novel eco-friendly electrospun polymer materials are promising candidates as active dressings for agricultural application to protect grapevine plants from the penetration and development of the two main fungal agents causing the esca disease.

V. DISSERTATION CONTRIBUTIONS

Innovative plant protection products based on polymers with antifungal activity against the fungi *P. chlamydospora* and *P. aleophilum* - the causative agents of the esca disease - have been created.

It was established that materials made of non-toxic, biodegradable and ecosafe polymers - poly(L-lactide), poly(3-hydroxybutyrate), polyvinylpyrrolidone, cellulose acetate, can be imparted antifungal properties by preparing mixed solutions with derivatives of 8-hydroxyquinoline (5-chloro-7-iodo-8-hydroxyquinoline, potassium 5-nitro-8-quinolinolate and 5-chloro-8-hydroxyquinoline) and of suspensions containing zinc oxide and their conversion into microfiber materials.

Methods have been developed for the preparation of mats with different composition, architecture and antifungal activity using electrospinning and electrospraying techniques.

The biologically active substances, derivatives of 8-hydroxyquinoline, included in the fibrous mats are in an amorphous state.

It has been shown that by using zinc oxide nanoparticles with a silanized surface and cellulose acetate, superhydrophobic materials (type "*on*"fibrous materials) can be successfully obtained.

VI. DIRECTIONS FOR FUTURE RESEARCH

Future research will focus on the creation of novel environmentally friendly agro-pharmaceuticals in which a biological agent will be incorporated into biocompatible and biodegradable polymer matrixes. In the systems to be obtained, the polymer carrier will play an active role by ensuring the viability of the biological agent during storage, and placed in contact with an aqueous environment will ensure its normal development. As a biological agent, a beneficial soil microorganism will be used - an antagonist of plant pathogenic microorganisms.

In this way, by combining the useful and valuable properties of biopolymers with those of the bioagent, innovative eco-friendly materials for agricultural purposes will be created, through which environmental protection will be achieved, the harmful effects of widely applied pesticides will be overcome, and protection of cultural plants from diseases caused by various phytopathogens will be ensured.

List of scientific papers and presentations

The dissertation summarizes the results reported in the following publications and scientific presentations:

PUBLICATIONS:

[P 1] Nachev N., Spasova M., Manolova N., Rashkov I., Naydenov M, Improving the water-repellent and antifungal properties of electrospun cellulose acetate materials by decoration with ZnO nanoparticles, *FIBRES & TEXTILES in Eastern Europe*, 29, 3(147), 40-45 (**2021**).

[P 2] Nachev N., Spasova M., Tsekova P., Manolova N., Rashkov I., Naydenov M., Electrospun polymer-fungicide nanocomposites for grapevine protection, *Polymers*, 13(21), 3673, 1-14 (**2021**).

[P 3] Ignatova M., Nachev N., Spasova M., Manolova N., Rashkov I., Naydenov M., Electrospun 5-chloro-7-iodo-8-hydroxyquinoline (Clioquinol)-containing poly(3-hydroxybutyrate)/polyvinylpyrrolidone antifungal materials prospective as active dressings against esca, *Polymers*, 14(3), 367, 1-17 (**2022**).

SCIENTIFIC PRESENTATIONS (the name of the reporting author is underlined):

Oral presentations:

[OP 1] N. Nachev, M. Spasova, N. Manolova, I. Rashkov, From electrospraying to electrospinning of cellulose acetate - influence of its concentration in the spinning solution. XII Spring Seminar for young scientists and PhD students in BAS "INTERDISCIPLINARY CHEMISTRY", organized by the Bulgarian Academy of Sciences and the Institute of Optical Materials and Technologies - BAS, 19-21 April 2019, Vitosha Creative House of BAS, Bulgaria.

[OP 2] N. Nachev, M. Spasova, N. Manolova, I. Rashkov, Composite cellulose acetate and nanosized ZnO based materials possessing potential applications in agriculture, Second interdisciplinary PhD forum dedicated to the 150th anniversary of the Bulgarian Academy of Sciences, August 29-31, 2019, Samokov Hotel, Borovets, Bulgaria.

[OP 3] N. Nachev, M. Spasova, N. Manolova, I. Rashkov, M. Naydenov, Polymer membranes from biodegradable polymer and chemical fungicide prepared by electrospinning, XXII National Textile Conference, 12-14 November 2020, National House of Science and Engineering, Sofia, Bulgaria.

[OP 4] N. Nachev, M. Spasova, N. Manolova, I. Rashkov, M. Naydenov, Polymer fibrous biomaterials with imparted antifungal activity, Third Youth Scientific Session "Biomedicine and Quality of Life", 2-3 December, 2021, Institute of Biophysics and Biomedical Engineering, Sofia, Bulgaria, online presentation.

Poster presentations:

[PP 1] N. Nachev, M. Spasova, O. Stoilova, N. Manolova, I. Rashkov, Bio-based composite membranes by combining electrospinning with electrospraying, 19th Symposium POLYMERS 2019, September 9-12, 2019, Pomorie, Bulgaria.

[PP 2] N. Nachev, M. Spasova, N. Manolova, I. Rashkov, M. Naydenov, Improving the water-repellent and antifungal properties of electrospun cellulose acetate

materials by decoration with ZnO nanoparticles, Eleventh Scientific Session “Young Scientists in the World of Polymers”, organized from Institute of Polymers – BAS, 10 September 2020, Sofia, Bulgaria.
[PP 3] N. Nachev, M. Spasova, N. Manolova, I. Rashkov, M. Naydenov, Polymer composite fibrous materials with imparted biological activity, Twelfth Scientific Session “Young Scientists in the World of Polymers”, organized from Institute of Polymers – BAS, 3 June 2021, Sofia, Bulgaria.
[PP 4] M. Ignatova, N. Nachev, M. Spasova, N. Manolova, I. Rashkov, M. Naydenov, Preparation, characterization and antifungal activity of new electrofibrous materials from poly(3-hydroxybutyrate) and polyvinylpyrrolidone, incorporating an 8-hydroxyquinoline derivative, Twelfth Scientific Session “Young Scientists in the World of Polymers”, organized from Institute of Polymers – BAS, 3 June 2021, Sofia, Bulgaria.
[PP 5] N. Nachev, M. Spasova, N. Manolova, I. Rashkov, M. Naydenov, Preparation and characterization of electrospun 5-chloro-8-hydroxyquinoline-loaded polyester membranes against Esca, Materials, Methods & Technologies, 23nd International Conference 19-22 August 2021 Burgas, Bulgaria.
[PP 6] N. Nachev, M. Spasova, N. Manolova, I. Rashkov, M. Naydenov, Fibrous polymer materials with antifungal activity for grapevine protection, Scientific conference INFRAMAT 2021, September 8-10, 2021, Riu Hotel, Pravets, Bulgaria.

AWARD
For the best poster presentation at the Twelfth Scientific Session "Young Scientists in the World of Polymers", 2021.

NOTED CITATIONS OF PUBLICATIONS INCLUDED IN THE DISSERTATION:
[P 1] Nachev N., Spasova M., Manolova N., Rashkov I., Naydenov M, Improving the water-repellent and antifungal properties of electrospun cellulose acetate materials by decoration with ZnO nanoparticles, *FIBRES & TEXTILES in Eastern Europe*, 29, 3(147), 40-45 (**2021**), *noted **1** citation*.
1. Aguda O., Lateef A., Recent advances in functionalization of nanotextiles: A strategy to combat harmful microorganisms and emerging pathogens in the 21st century (review), Heliyon 8(6) e09761, 1-17 (**2022**).
[P 3] Ignatova M., Nachev N., Spasova M., Manolova N., Rashkov I., Naydenov M., Electrospun 5-chloro-7-iodo-8-hydroxyquinoline (Clioquinol)-containing poly(3-hydroxybutyrate)/polyvinylpyrrolidone antifungal materials prospective as active dressings against esca, *Polymers*, 14(3), 367, 1-17 (**2022**), *noted **1** citation*.
1. Liu H., Jiang W., Yang Z., Chen X., Yu D.-G., Shao J., Hybrid films prepared from a combination of electrospinning and casting for offering a dual-phase drug release, *Polymers*, 14(11), 2132, 1-15 (**2022**).

PARTICIPATION IN SCIENTIFIC PROJECTS:
*Grant **KP-06-OPR03/2,** Funding: Bulgarian National Science Fund, “Design and methods for creation of innovative polymer composites with fungicidal activity against*

Phaeomoniella chlamydospora and Phaeoacremonium aleophilum“ from 14.12.2018 https://lbapproject2018.wordpress.com/
*Grant **KP-06-N39/13,** Funding: Bulgarian National Science Fund, “Preparation of innovative antimicrobial and antitumor electrospun materials from mucoadhesive and biocompatible polymers and chelating agents from the 8-quinolinol group” from 09.12.2019 https://electrospunchelating.wordpress.com/*