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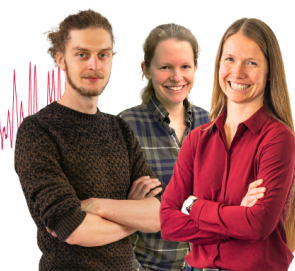
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Organic Nanoparticles from Lignocellulosic Agricultural Residues for Plant Protection Applications

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Abstract. Several aspects of agriculture industry can be made more innovative and efficient by relying on nanotechnology tools. Moreover, extraction of lignocellulosic fractions from agricultural residues and the following synthesis of organic nanoparticles for applications in plant disease management well fit the need of more sustainable plant protection strategies. A selection of the agro-forestry chains interesting for the Tuscia region has been performed, by identifying the most suitable residues for further valorization. Afterwards, different protocols of extractions including mechanical, chemical and enzymatic pretreatments have been tested in order to obtain organic materials suitable to be used as nanocarriers for green, efficient and innovative agrochemicals.

INTRODUCTION

Nanotechnology combined with circular economy concept represents a new, fundamental green alternative to deal with the challenges that the agricultural sector is facing today: increase in the agricultural products demand, climate change, urbanization, environmental issues like runoff and accumulation of pesticides and fertilizers [1]. The traditional agriculture system, inefficient and chemical-based, is going to miss these challenges and nanotechnology can be the answer to the failure of conventional methods by ensuring food security in a sustainable way. In fact, the last decades pointed out the great relevance to develop worldwide sustainable control strategies against plant pathogens and pests, by strongly reducing the use of chemicals. The success of organic productions and the request of the civil society has imposed a change of strategy for agrochemicals companies. Different organic materials are becoming of great relevance to be included in plant protection strategies, from the open field until to the

commercialization of fresh agro-foods: the use of organic compounds will help in reducing environmental and product damages at each production step [2].

This novel approach allows for example to obtain highly technological nanometric materials, such as nano-agrochemicals, starting from agro-industrial wastes. The residues of a supply chain may become part of the strategy for the sustainable growth and protection of the supply chain itself. Nanoscale structures like organic nanomaterials used to encapsulate and entrap agrochemicals such as fertilizers, pesticides, herbicides, plant growth regulators and other active substances, can be utilized in smart delivery of plant protection inputs, by ensuring the slow uptake of active ingredients and reducing the amount of agrochemical applications by minimizing the input and waste [3].

Today the combination of bio-based additives, as active ingredients, and functional nanostructures, as carriers, is one of the most promising alternatives to conventional plant protection strategies.

The aim of the paper is to give a first insight of the potentiality of cellulose nanocrystals (CNCs) in agriculture applications, such as in the plant protection sector, together with a practical example of circular valorization of lignocellulosic wastes obtained from many agri-food supply chains, selected by the University of Tuscia in the frame of the project PON NEMESI, by describing a possible method of cellulose extraction and CNCs synthesis, which is currently being studied and evaluated internally.

ORGANIC NANOMATERIALS FROM LIGNOCELLULOSIC BIOMASS

Lignocellulosic materials are natural, renewable, readily available, environmentally friendly, biodegradable, and inexpensive resources with a growing importance for the industrial sector. In comparison to synthetic materials, lignocellulosic materials present some advantageous characteristics: a) a wide variety of fillers worldwide extractable from forest and agro sources or wastes; b) low density, cost, and energy consumption; c) specific strength and modulus; d) reactive surfaces that can be easily modified and functionalized by different reactive groups; e) high applicability in composite and/or nanocomposite systems; and f) recyclability in comparison with other inorganic fillers [4].

Moreover, it is important to stress that lignocellulosic biomass consists of nanoscale building blocks that provide valuable properties to wood and other types of cellulosic and lignocellulosic biomaterials. Indeed, all the great properties mentioned could be useful in material science, especially when at the nanoscale. Consequently, lignocellulosic nanostructures, mainly cellulose- and lignin-based nanoscale materials, are gaining always more attention due to their renewable nature and the wide variety of sources available throughout the world, consisting mainly of wastes [5].

In particular, lignocellulosic agricultural residues represent an important resource with great opportunities and intrinsic potential: they can be handled as wastes, or they could be converted in high value-added compounds, following the circular economy concept. In such a case, the residual biomass obtained from pruning or other manufacturing processes can be valorized through extraction of organic compounds, such as cellulose and lignin. These organic fractions of lignocellulosic biomass can be further converted in their nanoscale form, exhibiting many more and exploitable features respect to their bulk counterpart, such as higher strength and stiffness, as well as a greater reactivity and an increased exchange surface (greater area/volume ratio), and sometimes also a direct antimicrobial activity.

In that specific case, lignocellulosic materials, and in particular cellulose and lignin, are being considered as potential nanocarriers for active ingredients or in combination in agri-food sector applications [6]. By focusing on plant disease management applications, these organic nanomaterials play an important role in decreasing toxicity and in turn in increasing the efficacy of pesticides [7]: the so-called “nanopesticide” formulations increase the solubility of poorly soluble active ingredient and help in releasing the active ingredient slowly. In general, one of the main advantages in using nanomaterials, directly or as carriers, rely on the fact that the reactivity at nanoscale is higher compared to their bulk counterparts, by allowing a lower quantity use of nanocides as well as an enhanced effect in crops protection [8].

Cellulose Nanocrystals: Nature and Applications

Cellulose is a polysaccharide found prevalently in plant organisms: it is one of the main fractions of lignocellulosic biomass. It is the most abundant organic compound on the Earth, with an estimated annual production of around 7.5×10^{11} tons [9]. The cellulose chemical formula is $(C_6H_{10}O_5)_n$: it consists of glucose residues, that form cellobiose units linked in linear chains up to 12,000 residues (Fig.1). In plant secondary cell wall, cellulose is found in the form of 30 nm diameter microfibrils parallel to each other, forming crystalline regions within which cellulose molecules are tightly packed, and amorphous or soluble regions, in which the molecules are less compact; however, these regions are staggered, making the overall cellulose structure strong [10]. For its high mechanical strength, cellulose has gained attention in view of industrial applications as a highly stable natural material [11].

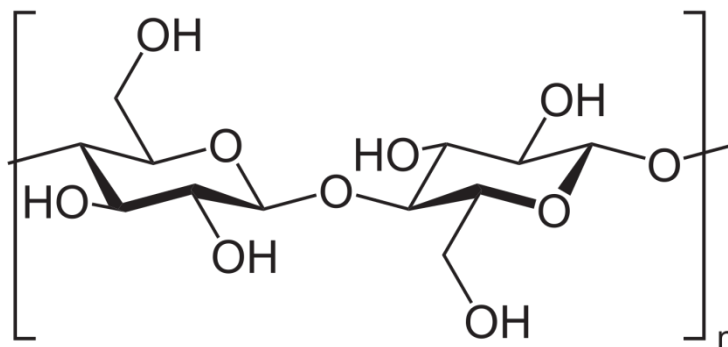


FIGURE 1. Structural formula of cellulose.

The high stiffness and strength of cellulose can be exploited also at the nanoscale level: its physical, chemical and biological properties, such as rigidity and stability, as well as the presence of hydroxyl groups prone to functionalization and the low toxicity, are intensified when converted to nanocellulose, offering the possibility of its use in the production of high-performance nanosystems and nanodevices [12]. Nanocellulose exists in several forms (fibrillated, nanocrystalline, and bacterial cellulose), classified based on the dimension and the preparation method applied. In particular, cellulose nanocrystals (CNCs) consist of rigid rod-like particles with a typical acicular structure, with diameter ranging from 1 nm to 100 nm and length from 100 to 500 nanometer (9). Cellulose nanocrystals have some different interesting properties: high stiffness, low density (approximately 1.57 g/cm^3), very low thermal expansion coefficient (estimated to be about 10^{-7} K^{-1}), high elastic modulus around 150 GPa; moreover in the last few years, attention has been posed on the surface modifications of CNCs, specifically on the abundant

hydroxyl groups present on their surfaces, and in particular some functionalizations including etherification, oxidation, esterification, polymer grafting, silylation, etc. These chemical functionalizations have been investigated to obtain better dispersion, introducing stable negative or positive electrostatic charges on the nanocellulose surface. All their interesting characteristics permit CNCs to be used as the reinforcement phase in thermoplastic and thermosetting matrices for many different applications. Moreover, they can be used in the biomedicine, pharmaceutical, and agricultural sectors for the release of specific drugs and active substances [13].

Many studies have been already conducted to assess the efficacy and the actual opportunity to use CNCs in different fields worldwide, and some of the different possibilities have been already tested internally by the groups of Fortunati and Balestra, especially in agricultural and food packaging applications.

CNCs, alone or in combination with poly (DL-Lactide-co-Glycolide) (PLGA) based nanoparticles (NP), were directly applied in plant protection treatment, in order to test their efficacy as nanocarriers in innovative plant protection formulations (Fig.2). They were investigated respect to both their bactericidal and phytotoxic effects: in such a case, Fortunati et al. tested the influence on the survival of the causal agent of bacterial speck disease (*Pseudomonas syringae* pv. *tomato*, Pst) and on plant development and damages, on tomato plants. The proposed nanocarriers resulted able to cover, by a uniform distribution, the tomato vegetal surfaces without any damage and allowing a regular development of the tomato treated plants: CNCs, with their typical acicular structures, were clearly visible on the adaxial surface of tomato leaves, and no visible effects on stomata and parenchyma cells appearance were detected (Fig. 2c, d—arrows indicate the stomata) [14].

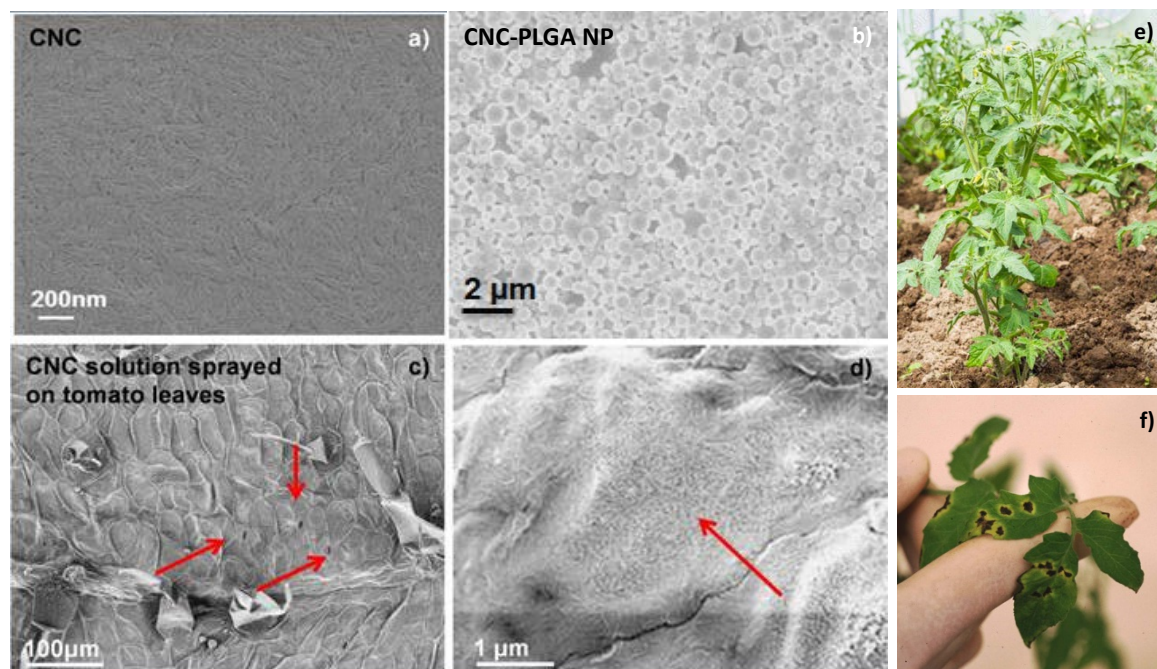


FIGURE 2. Morphological characterization of: CNCs nanoparticles (a), CNCs–PLGA NP (b), stomata and parenchyma cells (c) and adaxial surface (d) of tomato leaves when applied CNCs at pristine 0.5 %wt concentration by FESEM - red arrows indicate the stomata; tomato plant (e) and bacterial speck disease symptoms (f). Observations were taken from Fortunati et al. [13].

High performing cellulose nanocrystals were also extracted from *Actinidia deliciosa* pruning residues, with a protocol combining a bleaching treatment and an acid hydrolysis (Fig.3). The resultant CNCs were then used as reinforcements phases in poly(vinyl alcohol) (PVA) blended with natural chitosan (CH) based films and combined with carvacrol as active agent, among other formulations (Table1). Morphological and optical characteristics, mechanical response, thermal and migration properties, moisture content and antioxidant and antimicrobial assays were conducted, suggesting the possible application in industrial sectors. In particular, four known high virulent plant pathogenic bacterial strains, *Pectobacterium carotovorum* susp. *odoriferum* (Pco – CFBP 1878T), *Erwinia amylovora* (Ea – CFBP 1430), *Xanthomonas axonopodis* pv.*vesicatoria* (Xav – CFBP 3274), and *Xanthomonas arboricola* pv. *pruni* (Xap – CFBP 3894) were here considered for films antimicrobial assays. From tests with Pco emerged the efficacy of cellulose nanocrystals associated with carvacrol in the films, in fact the thesis subjected to this treatment did record a significantly lower bacterial population than the control. The reduction was quantified at 1 log unit; in fact at the time 12 h positive control measured population was 5.82×10^7 CFU/mL, while in PVA_5Carv_3CNC thesis the population was present at a concentration equal to 1.95×10^6 CFU/mL. Good results were similarly recorded by using all films containing cellulose nanocrystals; in fact these, compared with the respective waived cellulose nanocrystals, determined a lower bacterial population values (Fig.3d). In the case of Xav, the causal agent of tomato bacterial spot, the positive control at the time was 12 h measure a value of 6.15×10^6 CFU/mL: overall, it showed effectiveness in containing Xav growth by about 1 log unit of all the films used except for one containing only chitosan, which was even recorded a significantly higher population for the positive control (9.05×10^6 CFU/mL) (Fig.3e).

The inhibition of bacterial development detected suggests the protective function of the PVA-CH-CNC film against microorganisms contamination [15].

TABLE 1. Material formulations - from Luzi et al. [15].

Formulations	PVA (% wt)	CH (% wt)	Carv (% wt)	CNC (% wt)
PVA	100	-	-	-
PVA_10CH	90	10	-	-
PVA_5Carv	95	-	5	-
PVA_10CH_5Carv	85	10	5	-
PVA_10CH_3CNC	87	10	-	3
PVA_5Carv_3CNC	92	-	5	3
PVA_10CH_5Carv_3 CNC	82	10	5	3

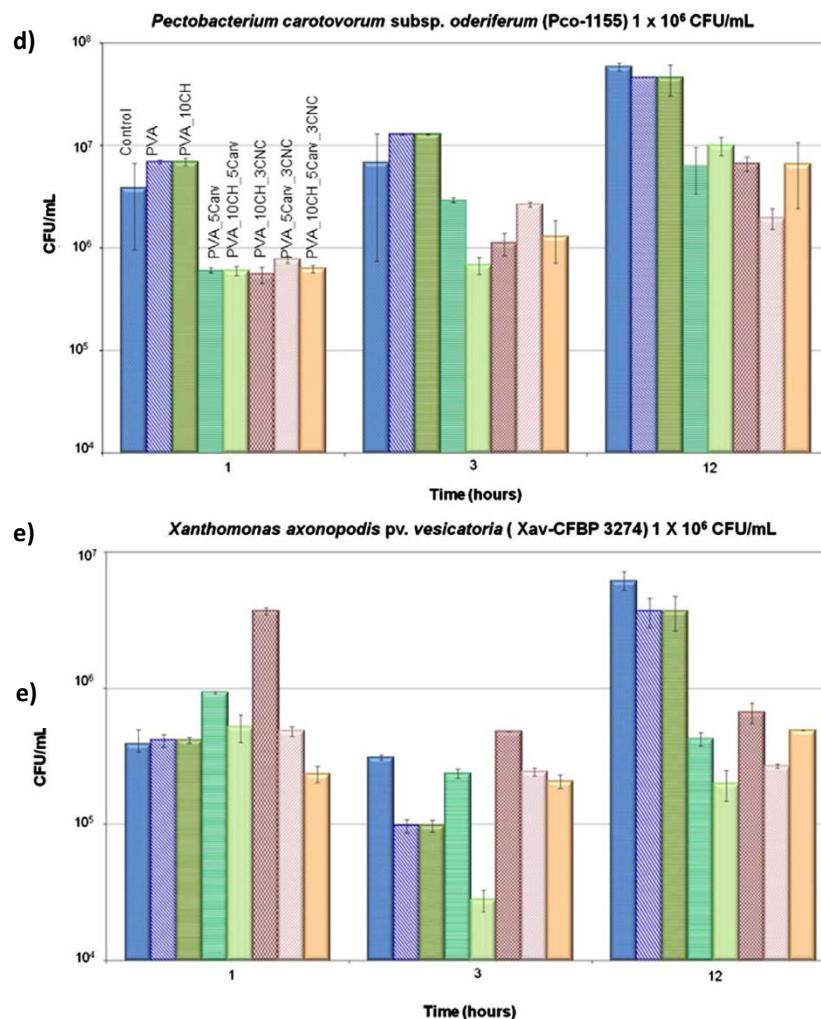
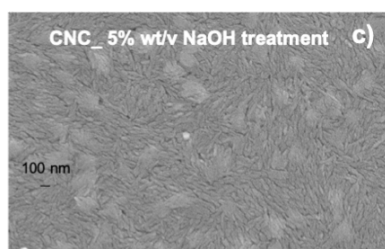


FIGURE 3. Visual image of Kiwi tree (a), visual image of pruned kiwi shoots (b) and FESEM investigation of CNCs extracted from pre-treated kiwi fibres with 5% wt/v of sodium chlorite (c). Antibacterial activities of PVA based formulations respect to *Pectobacterium carotovorum* susp. *oderiferum* (Pco – 1115) (d) and *Xanthomonas axonopodis* pv. *vesicatoria* (Xav – CFBP 3274) (e) multiplication. Pictures and data were taken from Luzi et al. [15].

CNCs Extraction: Supply Chain Revalorization for Plant Protection

With the aim to follow the concept of circularity, it is possible to obtain high performing cellulose nanocrystals directly from residues of pruning or manufacturing processes of the agro-industrial system. The first step consists in the selection of supply chains of interest, based on both the abundance of the biomass and on the relative amount of cellulose present into; different types of residues were also investigated in order to cover all the steps of the supply chain (harvest and pruning residues as well as fruit shells). Moreover, the crystallinity degree and the morphology of CNCs depend not only on the natural sources utilized as raw material, but both on preparation conditions and on the different parameters adopted during the extraction process, making tests and comparison of different feedstocks and extraction protocols even more important from an industrial point of view [16].

Figure 4 shows the supply chains selected by the University of Tuscia in the frame of the project PON NEMESI and Table 2 summarizes the matrices taken in consideration as interesting biomass both for their presence on the territory of the Tuscia region (Lazio, Italy), and for the relative abundance of compounds of interest (cellulose and lignin).



FIGURE 4. Olive tree (a), Kiwi tree fruits and shoots (b), sunflowers (c), hazelnut fruits (d), grapevine (e) and wheat spikes (f).

TABLE 2. Lignocellulosic feedstock and relative abundance of organic compounds of interest [17].

Lignocellulosic feedstock	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Olive pruning residues	40-55	25-40	15-25
Kiwi pruning residues	40-55	25-35	25
Sunflower stems	45-55	20-25	20-28
Nut shells	25-30	25-30	30-40
Vine pruning residues	25-35	15-25	15-20
Wheat straw	25-35	40-50	15

Each type of biomass needs to be pretreated with a combination of mechanical and chemical steps in order to make the cellulose accessible to the acid for the final CNCs synthesis.

CNCs Synthesis: Chemical Protocol and Enzymatic Pre-treatment

The authors are evaluating and implementing protocols for matrix pre-treatment, cellulose extraction and CNCs synthesis from the lignocellulosic biomass obtained from all the selected supply chains. In general, especially for the pruning residues, the protocol is the following (Fig.5a). Pruning shoots are firstly washed in distilled water several times in order to remove the powder and environmental contaminations. Subsequently, the pruning residues are treated for 72 h at room temperature (RT) with 1%w/v of NaOH solution and then for 2 h at 98°C with 5%w/v of NaOH solution to remove the external lignin components. The lignocellulosic materials are then mechanically retting and chopped in order to obtain individualized fibers (length around 10–15 mm) and the fibers are dried at 60°C for 24 h. Afterwards, the bleaching treatment is performed: the fibers are bleached with 5% wt/v of sodium chlorite (NaClO_2) (liquor ratio 1:50) [18], by boiling the solution with the fibers inside for two hours. The solution pH must be adjusted to 3.5-4 by means organic acids before the addition of the biomass. At the end of bleaching treatments the materials are washed several times and treated with sodium bisulphate solution at 5% wt/v for 30 min at RT (liquor ratio 1:40), washed again with deionized water, treated with NaOH solution at 17.5% wt/v for 20 min at RT (liquor ratio 1:50) and then finally dried at 60°C for 24 h. Cellulose nanocrystals are then prepared from this bleached material by means of acid hydrolysis, performed with 64% (wt/wt) sulphuric acid at 45°C for 30 min [19]. Cellulose suspension is made precipitate for 2 days and then dialyzed with nitrocellulose membranes against distilled water until the pH becomes 6-7. The resultant cellulose nanocrystal aqueous suspension is ultrasonicated by means of a tip sonicator. The final yield must be calculated as % of initial weight of the used raw material, together with the whole energy and chemical consumption in order to evaluate the possible scaling-up and the industrial applicability of the process.

Variations of the protocol may be necessary for the processing of matrices with characteristics other than pruning residues, such as for example nut shells and wheat straw. These latter materials present a waxy fraction that must be removed to allow the penetration of the chemical solvents to the matrix, by using for example a combination of benzene-methanol or toluene-ethanol prior to the bleaching step. Moreover, in order to make the matrix more accessible, the nut shells biomass must be grinded and sieved at the beginning of the process.

The chemical route can be implemented with the addition of an enzymatic pretreatment (Fig.5b). Enzymatic bioprocessing methods are of interest as an alternative to the traditional stand-alone or combined chemical, biological and mechanical processing steps. This is due to the potential for reduced energy and chemical usage, as well as high selectivity of these biocatalysts [20].

The enzymatic cocktail used in that case is a mix of cellulases (endo- and exo-, together with beta-glucosidases) and hemicellulases. The enzymatic hydrolysis is performed in agitation with optimal working conditions (50°C, pH 5) in a 50 mM citrate buffer and a solid-volume ratio 1:10. The optimal dosage of the enzymatic cocktail must be evaluated for each type of biomass by performing a dosage curve, since the different features of the matrices make the biomass more or less accessible to the enzyme. In general, a good dosage could be around 3% w/w enzyme/oven dried pulp, and the overall hydrolysis time can be modulated to ensure the depolymerization of the amorphous cellulose region only.

The use of an appropriate enzymatic cocktail can be useful to depolymerize the amorphous region of the cellulose, making the crystalline one more accessible to the sulphuric acid. In this way, the acid percentage used for the final CNCs synthesis can be decreased from 64% to 62% (wt/wt) [21], by obtaining even better quality (with a higher crystallinity index) cellulose nanocrystals. In fact, in literature isolation of CNCs has been carried out by diverse methods, even though characterized by low yields and unstable suspensions, reducing their economic and environmental efficiency. Actually, studies on cotton linters demonstrated that the combination of an enzymatic treatment with sulfuric acid hydrolysis could increase the yield of CNCs isolation while influencing other properties and leading to well-stable suspensions of electrically charged nanoparticles [21]. Hence, the introduction of biotechnology would improve the efficiency of this isolation as well as it would permit to reduce the environmental impact of sulfuric acid hydrolysis: the use of milder working conditions is preferable for a greener and more sustainable process.

The enzymatic route can be exploited also in combination with mechanical and physical pretreatments, avoiding any step involving chemical solvents, to make the overall process greener and solvent-free. In this latter case, final yields and energy consumption considerations must be done in order to assess the industrial viability of the process.

The resultant CNCs must be evaluated for their yield, quality, chemical and biological characteristics.

The effectiveness and the purity of the extraction process can be assessed by studies of microscopy and spectroscopy, together with chemical and morphological features. Transmission electron microscopy (TEM) and/or scanning electron microscopy (SEM) give direct multi-dimensional information about nanofibril morphology and structural properties [22]. The main difference between the two microscopy methods is that, as a result, TEM offers valuable information on the inner structure of the sample, such as crystal structure, morphology and stress state information, while SEM provides information on the sample's surface and its composition. Anyway, both methods can be used to assess the nano-size of the samples. In addition, dynamic light scattering (DLS) is used to obtain data about the mean size of all the particles in the samples. Microscopy in combination with the DLS results would be able to provide advanced insight to the morphology of the cellulose nanoparticles. An exact size to the decimal point is not realistic for nanoparticle size description; instead, ranges are of more importance (20). Fourier transformed infrared spectroscopy (FTIR) spectra of the cellulose nanoparticles produced are measured to have an idea of the chemical composition of samples: the different peaks are related to the presence of specific groups, by giving information about the purity of the CNCs synthesized [21].

The biological activity can be tested directly with *in vitro* or *in vivo* applications on plant, in order to assess CNCs phytotoxicity [14], and with *in vitro* and *in vivo* tests on different pathogens, to evaluate the possible antimicrobial activity [15].

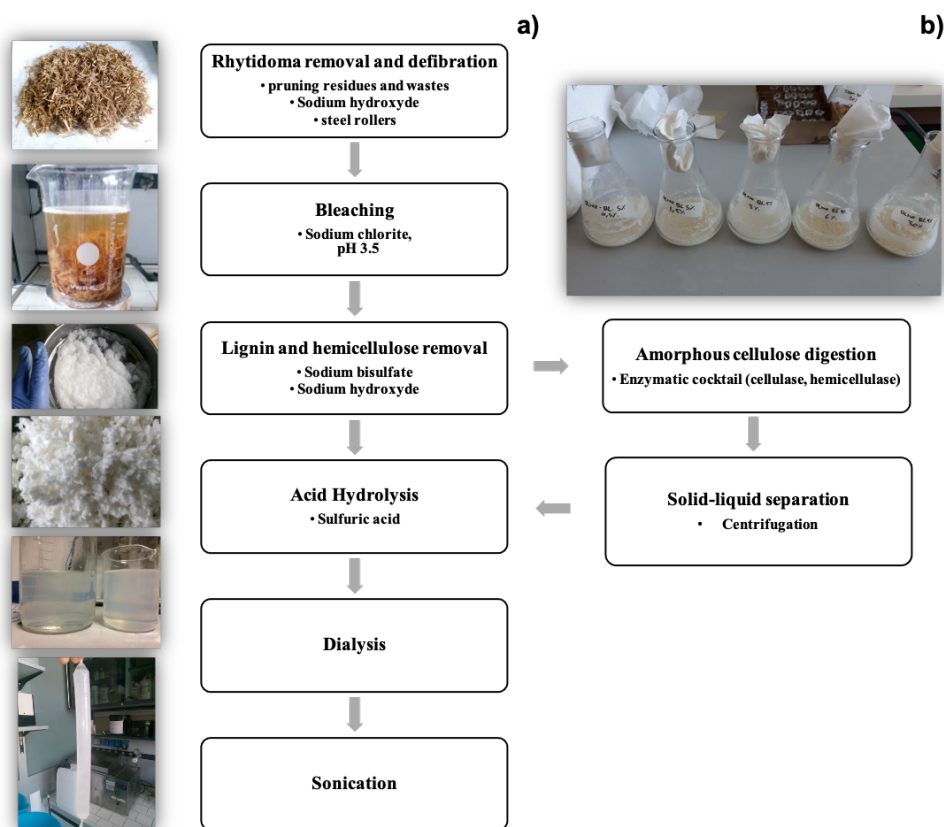


FIGURE 5. Chemical (a) and enzymatic (b) routes for CNCs synthesis from lignocellulosic biomass.

CONCLUSIONS

Plant protection research today is focusing on new strategies to counteract plant and crop pathologies, that are not relying on conventional, inefficient and environmental hazardous approaches. One possible aim is to obtain new formulations actually capable of counteracting pathogens, having a low environmental impact and reduced cost of production, being compatible with organic management systems. Nanotechnology can meet all these needs in a circular economy approach. The exploitation of lignocellulosic residues derived from many supply chains, for the extraction of valuable components, and the standardization of green and efficient protocols for the synthesis of cellulose nanocrystals from waste biomass, to be used as nanocarriers in natural-based agrochemicals, represent a worth circular approach, that can meet the idea of a sustainable plant protection. The research results today are promising but many efforts need to be done to reach an industrially relevant and economically feasible production.

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REFERENCES

1. Chen H, Yada R. Nanotechnologies in agriculture: New tools for sustainable development. [Trends Food Sci Technol](#). **22**(11), 585–594 (2011)
2. Fortunati E, Verma D, Luzi F, Mazzaglia A, Torre L, Balestra GM. Novel nanoscaled materials from lignocellulosic sources: Potential applications in the agricultural sector. [Handb Ecomater](#). **4**, 2657–2679 (2019)
3. Kannan M, Kolanthasamy E. Nanotechnological Approaches in Plant Protection. (2020)
4. Mohanty AK, Misra M, Drzal LT. Sustainable Bio-Composites from renewable resources: Opportunities and challenges in the green materials world. [J Polym Environ](#). **10**, 1–2 (2002)
5. Fortunati E, Yang W, Luzi F, Kenny J, Torre L, Puglia D. Lignocellulosic nanostructures as reinforcement in extruded and solvent casted polymeric nanocomposites: an overview. *European Polymer Journal*. (2016)
6. Yang W, Fortunati E, Dominici F, Giovanale G, Mazzaglia A, Balestra GM, et al. Synergic effect of cellulose and lignin nanostructures in PLA based systems for food antibacterial packaging. *Eur Polym J*. (2016)
7. Bhatia SC, Bhatia SC. Nanotechnology in agriculture and food industry. *Food Biotechnol*. 363–77. (2019)
8. Debnath N, Das S, Seth D, Chandra R, Bhattacharya SC, Goswami A. Entomotoxic effect of silica nanoparticles against *Sitophilus oryzae* (L.). *J Pest Sci*. (2004)
9. Habibi Y. Key advances in the chemical modification of nanocelluloses. *Chemical Society Reviews*. (2014)
10. Mäkelä MR, Marinović M, Nousiainen P, Liwanag AJM, Benoit I, Sipilä J, et al. Aromatic metabolism of filamentous fungi in relation to the presence of aromatic compounds in plant biomass. *Adv Appl Microbiol*. (2015)
11. Lavoine N, Desloges I, Bras J. Microfibrillated cellulose coatings as new release systems for active packaging. *Carbohydr Polym*. (2014)
12. Brinchi L, Cotana F, Fortunati E, Kenny JM. Production of nanocrystalline cellulose from lignocellulosic biomass: Technology and applications. *Carbohydrate Polymers*. (2013)
13. Fortunati E, Balestra GM. Lignocellulosic materials as novel carriers, also at nanoscale, of organic active principles for agri-food applications. In: *Biomass, Biopolymer-Based Materials, and Bioenergy: Construction, Biomedical, and other Industrial Applications*. (2019)
14. Fortunati E, Rescignano N, Botticella E, La Fiandra D, Renzi M, Mazzaglia A, et al. Effect of poly(DL-lactide-co-glycolide) nanoparticles or cellulose nanocrystals-based formulations on *Pseudomonas syringae* pv. tomato (Pst) and tomato plant development. *J Plant Dis Prot*. (2016)
15. Luzi F, Fortunati E, Giovanale G, Mazzaglia A, Torre L, Balestra GM. Cellulose nanocrystals from *Actinidia deliciosa* pruning residues combined with carvacrol in PVA/CH films with antioxidant/antimicrobial properties for packaging applications. *Int J Biol Macromol*. (2017)
16. Luzi F, Fortunati E, Puglia D, Lavorgna M, Santulli C, Kenny JM, et al. Optimized extraction of cellulose nanocrystals from pristine and carded hemp fibres. *Ind Crops Prod*. (2014)

17. Tayyab M, Noman A, Islam W, Waheed S, Arafat Y, Ali F, et al. Bioethanol production from lignocellulosic biomass by environment-friendly pretreatment methods: A review. *Applied Ecology and Environmental Research*. (2018)
18. Fortunati E, Luzi F, Puglia D, Petrucci R, Kenny JM, Torre L. Processing of PLA nanocomposites with cellulose nanocrystals extracted from *Posidonia oceanica* waste: Innovative reuse of coastal plant. *Ind Crops Prod*. (2015)
19. Fortunati E, Luzi F, Puglia D, Dominici F, Santulli C, Kenny JM, et al. Investigation of thermo-mechanical, chemical and degradative properties of PLA-limonene films reinforced with cellulose nanocrystals extracted from *Phormium tenax* leaves. *Eur Polym J*. (2014)
20. Bester LM. Development and optimisation of a process for cellulose nanoparticle production from waste paper sludge with enzymatic hydrolysis as an integral part. 50–70 (2018)
21. Beltramino F, Roncero MB, Torres AL, Vidal T, Valls C. Optimization of sulfuric acid hydrolysis conditions for preparation of nanocrystalline cellulose from enzymatically pretreated fibers. *Cellulose*. **23**(3), 1777–1789 (2016)
22. Desmaisons J, Boutonnet E, Rueff M, Dufresne A, Bras J. A new quality index for benchmarking of different cellulose nanofibrils. *Carbohydr Polym*. 174 (2017)