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Efficiency of lignin nanocapsules for delivering neem oil and capsaicin against pest insects: insights into the system *Eruca sativa* – *Plutella xylostella*†

Sara Falsini, *[a] Tommaso Nieri,[a] Alessio Papini,[a] Maria Cristina Salvatici,[b] Ali Abou-Hassan, [cd] Cristina Gonnelli [a] and Sandra Ristori *[e]

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In this paper, we report on the design, production and in depth characterization of nanoformulations based on kraft lignin for delivering neem oil and capsaicin as insect repellents. The procedure followed was aimed at establishing a protocol for scalable preparations, which can also ensure that the obtained dispersions are stable in water media, from where they can be administered by safe and easy routes (*e.g.* foliar spray). Lignin was initially dispersed in alkaline solution to obtain a concentration of 5% w/w. After oil addition in comparable proportion (4.5% v/v), the resulting dispersed aggregates were downsized by sonication. To increase the insect repellency effect, capsaicin was added to half of the samples by dissolution in the oil phase. Extensive structural characterization by DLS, electron microscopy and SAXS showed that all formulations contained well-defined particles with moderate polydispersity and globular shape, which tended to be more elongated in the case of lower starting pH and consequent lower surface charge of the particles. In all the samples, negative zeta potential values were measured, thus ensuring good stability by electrostatic repulsion. These findings represent a favourable premise for applications, since one possible drawback in the production of dispersed systems from natural sources is the ill-defined nature of the ensuing formulation, often showing thread-like interconnected structures coexisting with a small fraction of discrete objects, which can impart poor stability. The potentiality of the present formulations as insect repellents was tested on *Eruca sativa* plantlets against larvae of *Plutella xylostella* with encouraging results.

Environmental significance

In the last 50–60 years, pesticides have caused heavy pollution. Nanotechnology based on natural agents can help environmental preservation. For this purpose, micro and nano-carriers for bioactive principles have attracted attention. In particular, lignin formulations appear to be an appropriate choice, due to low cost and biocompatibility. We prepared lignin nanoassemblies to encapsulate plant secondary metabolites (neem oil and capsaicin), which suffer from low stability. Our protocol yielded globular aggregates, thus maximizing the surface available for targeted release. Extensive characterization gave a comprehensive picture of the structure and surface charge. A preliminary application was carried out against *Plutella xylostella* which shows resistance to standard pesticides. This work represents a case study of how nanocarriers from natural sources may ensure sustainable plant protection.

Introduction

Over the past 50–60 years, pesticides have enabled feeding the worldwide population at levels that prevented millions of people from starving every year. However, the growing production and distribution of foodstuffs have led to the excessive use of synthetic pesticides, causing major pollution of the ecosystem. In this context, crop protection by natural agents is becoming important to achieve far reaching aims such as the Sustainable Development Goal 2 (or the end of hunger) issued by the Agenda 2030 of the United Nations.[1]

[a] *Department of Biology, University of Florence, Via Micheli 1-3, Florence 50121, Italy. E-mail: sara.falsini@unifi.it*
[b] *Institute of Chemistry of Organometallic Compounds (ICCOM)-Electron Microscopy Centre (Ce.M.E.), National Research Council (CNR), Via Madonna del Piano n. 10, 50019 Sesto Fiorentino, Firenze, Italy*
[c] *CNRS, Physicochimie des Électrolytes et Nanosystèmes InterfaciauX (PHENIX), Sorbonne Université, F-75005 Paris, France*
[d] *Institut Universitaire de France (IUF), 75231 Cedex 05, Paris, France*
[e] *Department of Chemistry "Ugo Schiff" & CSGI, University of Florence, Sesto Fiorentino (FI), 50019, Italy. E-mail: sandra.ristori@unifi.it*
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Lately, many agricultural protocols have been modified in order to employ eco-friendly and innovative strategies such as sustainable nanotechnology. A sector where the principles of nanoscience can find a major role is the design of vectors for delivering natural compounds, such as hormones, pesticides, and fertilizers.[2–4] In fact, it has been shown that nanodelivery is able to increase the bioavailability of active principles by favouring the penetration into plant tissues. This also allows employing minimal effective doses[5] and reducing the amount of organic solvents that are sometimes used for the administration of poorly water-soluble molecules.[6] Natural bioactive compounds are more eco-friendly than synthetic molecules, since they are generally less toxic for humans.[7] Indeed, a fraction of pesticides sprayed aerially fail to reach the target, leading to hazards for non-target organisms and workers.[8]

Among possible vectors for bioactive compounds, large scale applications and polymeric micro and nano-aggregates have gained attention and, in particular, lignin-based nanoformulations appear an appropriate choice, due to their low cost and good biocompatibility.[9–12]

Fisher *et al.*[13] have loaded lignin nanocarriers with the fungicide pyraclostrobin to treat infected plants. Following a different approach, Peil *et al.*[14] have proposed the masking of antagonistic fungi, such as *Trichoderma reesei*, by using lignin nanocapsules against grapevine diseases. In a previous work, we used lignin nanocapsules for delivering gibberellic acid, to *Eruca sativa* and *Solanum lycopersicum* seeds[15] to promote germination, showing that by this route the hormone molecules were able to enter the seedling tissues. We have also prepared lignin–tannin mixed nanovectors to enhance the potentiality of natural pesticides against *Phaeoacremonium minimum.*[16] Lignin-based nanoparticles can be filled with hydrophobic compounds (oils) to be used in the administration of water insoluble bioactive compounds in a finely dispersed form.[17,18] Furthermore, encapsulation of oils into vectors can hinder the release of the cargo in the environment. Here, the main focus is the in-depth physico-chemical and morphological characterization of model formulations to be applied in agriculture.[19] Obtaining insights into the process of nanoparticle formation and stabilization helps to rationalize the synthesis and to find standardized protocols for obtaining tuneable carrier systems. This also helps to overcome problems that can be encountered when natural mixtures are used, *i.e.* the risk that heterogeneities in the composition are reflected into marked structural polydispersity. This is usually responsible for the occurrence of some very large particles (or even worm-like assemblies), with a consequent smaller surface/volume ratio and with the possibility that particle entanglement leads to loss of stability. The aim of this work was therefore to elucidate the properties of lignin nanocapsules containing neem oil and capsaicin as natural repellents.[20] Although their costs are relatively high, in comparison to chemical pesticides, the use of lignin nanocarriers could reduce the amount of needed neem oil and capsaicin, as observed with chemical pesticides.[13] Hence, this method could provide a less expensive alternative, especially if applications concern large scale systems, in which the cost of plant-derived pesticides is one of the possible limits to their use.[21]

Neem extracts are easily degraded and leached to soils after administration with conventional methods.[22,23] So, their absorption by plants can be more efficient if these disadvantages are reduced through encapsulation into the protective shell of a polymeric inert material. In particular, we were able to obtain solutions of globular aggregates rather than wires of lignin filled with oil, which allows us to maximize the surface available for targeted release. Moreover, since prolonged shelf life can be assured by electrostatic repulsion among nanoparticles, we brought lignin to its deprotonated state by carrying out the initial solubilization step at basic pH.[6,24,25] As the target plant with widespread use and commercial interest, we selected *Eruca sativa* L. (Brassicaceae). Preliminary results on the efficacy of these formulations as repellent agents were obtained against *Plutella xylostella* L. (Lepidoptera: Plutellidae), an insect commonly known as "cabbage moth", since its larvae feed on vegetative tissues of crops from the Brassicaceae family.[26,27] Our results show that novel carrier systems have good potential to address the problem of insecticide resistance by this plant pest while also suggesting wider application.

Experimental

Materials

Kraft lignin (alkali lignin, low sulphur, CAS number 8068-05-1) and acetone were purchased from Sigma-Aldrich and stored at room temperature (25 °C). KOH was provided by Sigma-Aldrich. Milli-Q filtered water was obtained from a Millipore system (20 MΩ cm at 25 °C). Capsaicin was provided by Sigma-Aldrich and the stock powder was stored at +4 °C. Neem oil was provided by Bio A.L.T. srl (Milan, Italy). *Eruca sativa* Mill. (Brassicaceae) seeds were provided by Blumen Group S.p.A. (Milan, Italy). The topsoil was purchased from COMPO Italia Srl (Ravenna, Italy).

Preparation of lignin nanocapsules

Capsaicin was dissolved in acetone at a concentration of 0.15 mg mL^{-1} and mixed with neem oil, at a 1 : 1 (v/v) ratio. 300 μL of this oil/acetone solution was added, dropwise and under magnetic stirring, to 3 mL of aqueous lignin. Then, high power sonication was applied to emulsify the oil phase in the lignin solution and to facilitate capsaicin uptake in the nanocapsules. For this purpose, a Sonopuls Bandelin was used at 95% of power, for 180 s (1 s pulse on and 0.5 s pulse off). The samples were dispersed finally in water. The final concentrations of capsaicin and neem oil were 5.3–6.8 × 10^{-3} mg mL^{-1} and 4.5 %v/v, respectively.

For the sake of clarity, the samples investigated in this study are listed in Table 1, where the composition and starting/final pH values are likely indicated. The final pH of the nanoformulations was determined by neem oil

Table 1 Sample name and composition, pH solution for dissolving the 5% lignin, and final pH of the formulations

Sample name	Composition	pH of the starting KOH solution	pH of the final formulation
N13	Lignin NCs and neem oil	13.5	11.0
NC13	Lignin NCs and neem oil + capsaicin	13.5	10.5
N11	Lignin NCs and neem oil	11.5	7.9
NC11	Lignin NCs neem oil + capsaicin +	11.5	6.4

encapsulation. Neem oil and capsaicin alone were also tested as water dispersions, and the results obtained with these heterogeneous formulations (macro-emulsions) are reported in ESI† 1 and 2.

The quantification of loaded capsaicin was determined using a UV/Vis spectrophotometer after NC disruption with CH_2Cl_2, followed by solvent evaporation and redissolution in ethanol (Fig. S1 and S2†). The absorption was measured at 288 nm and gave loading rates in water solution of 72% and 88% in NC11 and NC13, respectively.

Dynamic light scattering (DLS) and zeta potential (ZP)

DLS experiments were performed on a Zetasizer PRO Red Label (Malvern Panalytical Co., Ltd., Malvern, UK), equipped with an He–Ne 633 nm, 4 mW laser and backscattering optics at a 173° detection angle. Each measurement was averaged over 11 runs and taken in duplicate. Samples were diluted at 1 : 200 with MilliQ water to adjust optical density.

Zeta potential measurements were performed with a Zetasizer PRO Red Label (Malvern Panalytical Co., Ltd., Malvern, UK). Samples were diluted at 1 : 100 with MilliQ water. Each reported ZP value was averaged over 3 runs recorded at 2 minute intervals from one another.

Electron microscopy

SEM observations were performed using a Gaia 3 (Tescan s.r. o, Brno, Czech Republic) FIB-SEM (focused ion beam-scanning electron microscope). The electron beam used for SEM imaging had a voltage of 10 kV and operated in high vacuum and with a secondary electron detector at the CEME – Centro di Microscopie Elettroniche "Laura Bonzi", CNR Research Area (Florence, Italy).

Samples were deposited on a stub, dried, and then coated with an ultrathin coating of gold to enhance the contrast thanks to the presence of an electrically conductive material.

For TEM observations, the different aqueous suspensions of capsules were diluted in Milli Q water to obtain the final faint suspension. A drop (10 μL) of each solution was deposited on carbon coated grids, left to dry and imaged by transmission electron microscopy using a JEOL-1011 microscope at 100 kV.

Small angle X-ray scattering (SAXS) experiments

SAXS experiments were performed at the high brilliance SAXS beamline ID02 of the European Synchrotron Radiation Facility (ESRF), Grenoble, France. The wavelength of the incident photons was λ = 1 Å. The range of scattering vector $\boldsymbol{q}$ was 0.2–0.63 × 10^{-4} Å $^{-1}$, with a sample-detector distance ranging from 1 m to 30 m.

$\boldsymbol{q}$ is defined:

$$\boldsymbol{q} = \frac{4\pi}{\lambda}\sin\frac{\theta}{2}$$

where θ is the scattering angle and λ is the X-ray wavelength (= 0.1 nm).

Samples were diluted at 1 : 10 in Milli Q water and they were placed in quartz capillaries of 1.5 mm diameter. The temperature was 24 °C.

The SAXS intensities of the NCs were fitted using SAS view.

Evaluation of antifeedant activity

Eruca sativa Mill. plants were seeded in pots (diameter 6 cm). After 50 days from the germination with the development of the first true leaves, the plants were sprayed with the formulation of 1 : 10 diluted in a final volume of 2 mL (Fig. S3†). 18 plantlets were left under control conditions and each of the other 18 seedlings was exposed to 10 larvae of *Plutella xylostella* placed on the pot soil.

The control plants were treated with deionized water under the same conditions. The experiments were conducted in triplicate at 25 °C. A digital camera, placed at a distance of 15 cm from the pots, was used to take pictures of the three replicates of each treatment at different time steps immediately before the spray administration (time = 0) and after 24 h, 72 h, and 7 days after the treatment (time = 1, 3, and 7). The areas (cm^2) of the aerial parts including leaves and stems were measured with ImageJ v.1.54 software.[28]

Results

Lignin is sparely soluble in water media at low and intermediate pH values, but can be dissolved in alkaline solutions,[29,30] and thus as beforehand characterization, we carried out the acid–base titration of the kraft lignin specimen used in this work. We found that despite the occurrence of more than one type of deprotonable group, such as phenolic hydroxyls, aliphatic hydroxyls and benzyl alcohols in its molecular structure, the titration curve showed only a single equivalence point, as indicated by the sharp pH inflection in Fig. S4.† Following this result and to investigate the effect of deprotonation on the formation of nanoparticles, we dissolve lignin in alkali (KOH) solution at two different pHs, namely 11.5, *i.e.* just before the onset of

the plateau, and 13.5, *i.e.* at the plateau level. In both cases, the lignin concentration was set at 5% w/v.

Structural characterization and surface charge

DLS showed that the particles present in lignin–neem formulations were definitely submicron in size, having a *Z*-average diameter of 280–360 nm in intensity, with slightly lower values measured in capsaicin-loaded samples (Fig. 1). This evidence, joint with a high polydispersity index (PDI 0.4–0.6), indicated that the size distribution was broad and that, possibly, the equivalent sphere model applied by DLS cumulant analysis could only give an approximate representation of the particle structure.

A more detailed investigation was performed by filtering the samples through 0.45 μm pore diameter polycarbonate membranes. The DLS data obtained after filtration are shown in Fig. 2A and B. Indeed, in all the samples, objects with different size distribution could be evidenced. Subsequent ultracentrifugation (15′ three cycles at 16 100*g*) confirmed that a fraction of objects, with a diameter less than 100 nm, was present in solution (Fig. 2C and D). These particles could be due to free fragments of lignin, whose % in mass was slightly larger than neem oil.

The precise morphological properties of the NCs were investigated by Electron Microscopy (SEM and TEM), showing that both spherical and elongated aggregates were present in samples prepared at starting pH 11.5, while only globular particles (with varying sizes) were observed in samples prepared at starting pH 13.5. Typical SEM and TEM micrographs for these two series of samples are reported in Fig. 3. Overall, the morphology of the particles in plain and loaded samples appeared similar.

All nanoformulations showed remarkable shelf stability at 20 °C and this was ascribed to their negatively charged surface, as measured by zeta potential (Fig. 4). In particular, NCs prepared at starting pH 11.5 did not show any visible coalescence/precipitation for at least 2–3 weeks, while NCs prepared at pH 13.5 were stable for more than 1 month, in agreement with zeta values of these two-sample series. The variation in surface charge induced by the starting pH confirmed the significance of the chosen pH levels from the titration curve.

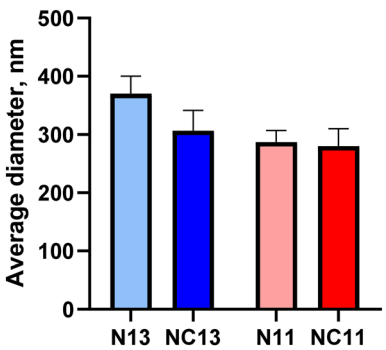

Fig. 1 Average NC diameters obtained by DLS measurements.

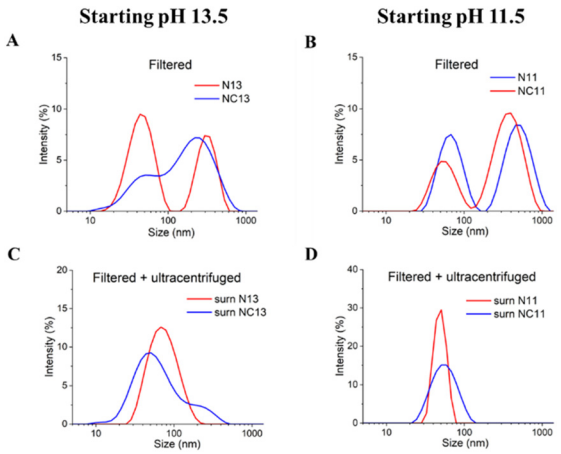

Fig. 2 DLS intensity profiles of lignin NCs prepared at starting pH 13.5 (left panel) and 11.5 (right panel) after filtration with 0.45 μm polycarbonate membranes (A and B) and ultracentrifugation at 14 000 rpm (three cycles for 15′) (C and D).

SAXS analysis

Fig. 5 shows the experimental and fitted SAXS data for N13 (Fig. 5A) and N11 (Fig. 5B). The intensity profiles decayed with large shoulders and no clear peaks, indicating that slightly different contributions superimposed to give the overall signal. Capsaicin loading did not alter the main features of the SAXS profiles. The SAXS curves of NC11 were simulated by considering core–shell cylinders and core–shell spheres, whereas only spherical aggregates were taken into

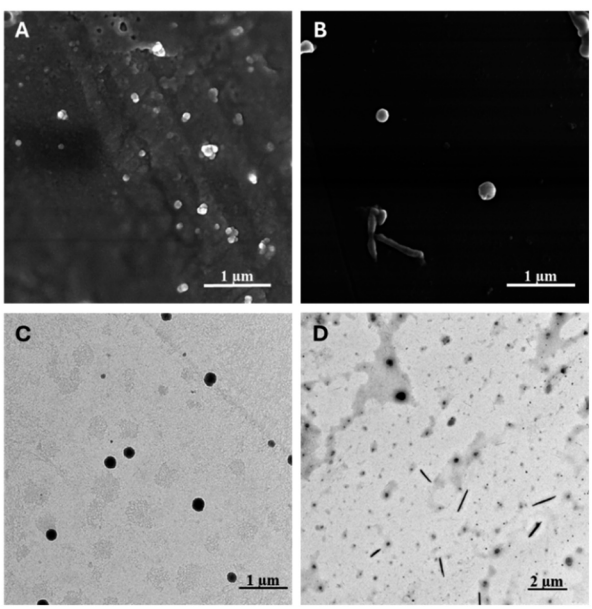

Fig. 3 SEM (A and B) and TEM (C and D) representative micrographs of N13 (A and C) and N11 (B and D) samples.

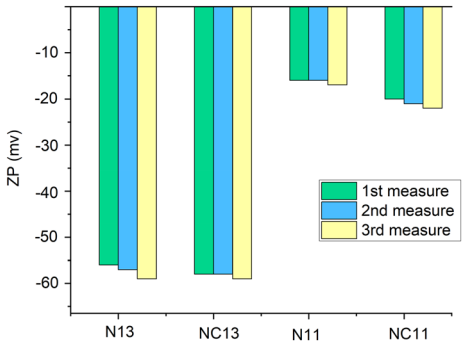

Fig. 4 Zeta potential of plain and capsaicin-loaded nanoformulations.

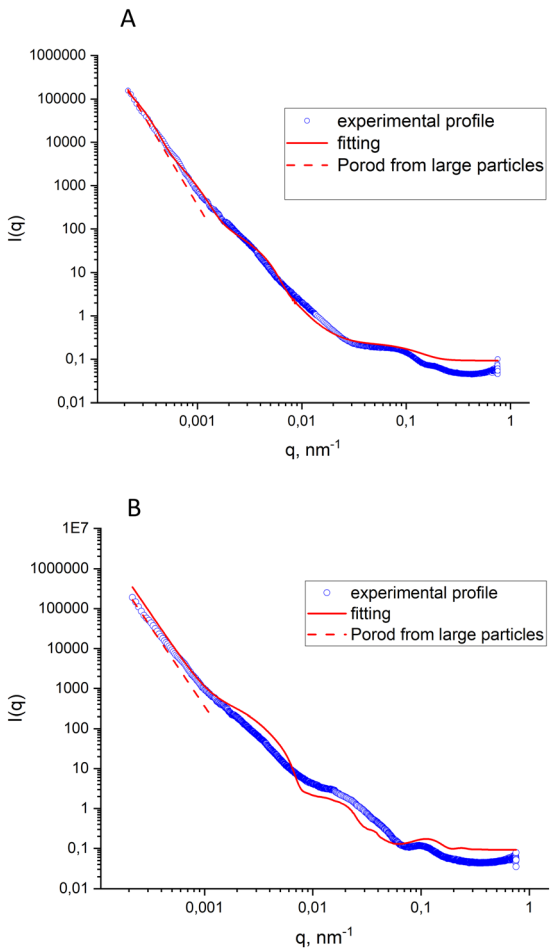

Fig. 5 Representative SAXS intensity profiles of N13 (A) and N11 (B). Blue circles: Experimental data; continuous red lines: best-fitting obtained with the contributions shown in Fig. S5;† dashed red line: q^{-4} decay due to large (≈1 μm) scattering objects.

account for samples prepared at starting pH 13.5. The contribution of elongated objects used for fitting the SAXS diagrams of the systems prepared at starting pH 11.5 was consistent with the microscopy data. In all cases, a q^{-4} trend was found at low q values and was interpreted as the Porod "tail" of large scattering objects (about 1 micron). This contribution was probably not influential in numerical terms, but it was visible due to its huge scattering intensity. The best fit curves in Fig. 5 were intentionally not smoothed with more polydispersity to evidence the capturing of different bumps in the experimental profiles.

Evaluation of antifeedant activity on *Eruca sativa*

The average daily increase in shoot growth was affected by the treatment at different intervals, after spray

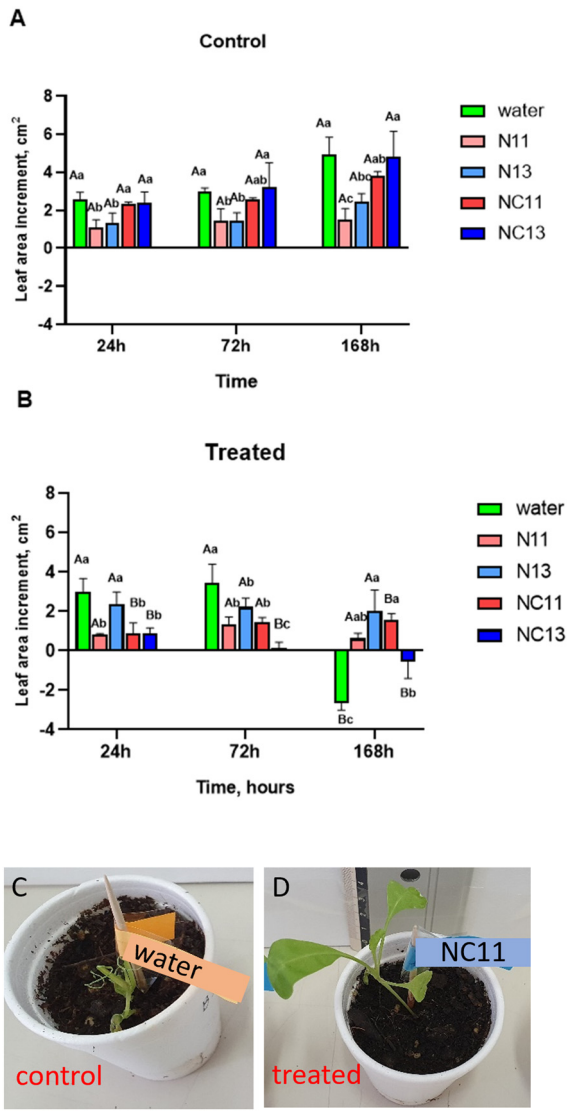

Fig. 6 Leaf area increment (cm^2) without any disturbance (A) and in the presence of parasites (B). Capital letters indicate significant differences (one-way ANOVA, at least $p < 0.05$) among the mean values of the control without any disturbance and samples treated with parasites within each sampling time (24 h, 72 h, and 168 h after spray administration). Lower case letters indicate significant differences (one-way ANOVA, at least $p < 0.05$) among the mean values of the leaf area increment obtained in water and after nanoformulation exposure within each sampling time (24 h, 72 h and 168 h after spray administration). (C) Example of the control plant and (D) example of the plant treated with NC11.

administration in control samples (Fig. 6A) and in the presence of *Plutella xylostella* larvae (Fig. 6B).

In particular, in the control samples after 24 h from the initial treatment, NC11 and NC13 exposed plants developed an increment in the leaf area similar to the treatment with simple water. A lower increase in growth was induced by the nanoformulations with only neem oil and without capsaicin. After 72 h, NC11 exposure started to affect negatively the plant growth as well as the N11 and N13 samples. A different trend was obtained with NC13 treatment. In this case, the increase in leaf area (4.8 cm^2 ± 1.4) was similar to the control (4.9 cm^2 ± 0.9). The administration of NC11, N13 and N11 promoted an increase in the leaf area of 3.8 cm^2 ± 0.2, 2.4 cm^2 ± 0.5 and 1.5 cm^2 ± 0.6, respectively. Neem oil and capsaicin alone were also tested as water dispersions, but the corresponding data are not reported here. In fact, applying these macro- (and heterogenous) emulsions failed to give reproducible results, since the sprayed solution could not be evenly distributed on the plant leaves.

In the presence of insects, after 24 h, N13 exposed plants showed an increase in the aerial part similar to the water (Fig. 6C), whereas the leaf area increment was smaller for the other treatments. After 48 h, the behaviour was similar to that after 24 h except for the plant treated with NC13 where a lower increment was observed. After 168 h, N13 and NC11 (Fig. 6D) represented the two treatments with the highest increment in the leaf area, considering that the shoots exposed to simple water and NC13 were completely eaten by *P. xylostella* larvae.

Discussion

The characterization results reported in the previous section show that lignin nanocapsules were successfully obtained in aqueous media. These formulations can be applied by spraying on plant leaves, which is a simple and convenient administration route for medium and large-scale applications. Neem oil was loaded in the hydrophobic core at the final concentration of 4.5% v/v.[31] Capsaicin was also encapsulated with rates in the range of 70–90% reaching a final concentration in the range of 5.3–6.8 × 10^{-3} mg mL^{-1}. These values are not attainable without an appropriate carrier, indicating that the present systems represent a viable route for treating pests with natural (but poorly bioavailable alone) oils and polyphenols.

From DLS measurements, the nanoaggregates showed dimensions in the range of 300–400 nm, which are suitable for delivery purposes.[32,33] The measured PDIs were relatively high with values between 0.4 and 0.6, suggesting the polydispersity of nanoparticles with the presence of more than one population in solution. Indeed, filtration revealed lignin systems with a size of 100 nm or less, while from electron microscopy and SAXS diagrams, larger particles were also evidenced. This confirmed that a plurality of aggregates was present in all NC formulations. Such interpretation was in agreement with previous studies where lignin nanoparticles prepared under similar conditions were described.[34,35]

In this work, in-depth physicochemical characterization allowed us to rationalize the behaviour of nanoformulations prepared at different starting pH values of the alkali solutions in which lignin was solubilized. First of all, it was evidenced that the starting pH had an effect on the surface charge, though the measured ZP values remained negative in all cases. In general, negative ZPs are advantageous for nanocarriers since this property is associated to stable solutions and minimal effects toward the target organisms.[36] Moreover, differences in morphology were evidenced between NC11 in and NC13 by electron microscopy in agreement with the structural data obtained from SAXS profiles. In particular, NC11 showed the presence of both cylindrical and spherical objects, whereas only spherical aggregates were observed for samples prepared at starting pH 13.5. We can thus speculate that a lower surface charge density promotes the elongation of the particles and that the corresponding, slightly larger interface is responsible of more efficient cargo release.

Finally, from preliminary results on the protection against *Plutella xylostella*, we were able to assess that neem oil and capsaicin carried by lignin submicron capsules efficiently exert their action on the *Eruca sativa* plantlet and that this effect persisted for seven days avoiding the loss of leaves.

Conclusions

The ongoing search of appropriate submicron carriers for natural compounds has opened new routes to develop innovative agro-technological products.

In this work, stable formulations were obtained by choosing right pH values to fully dissolve kraft lignin and obtain negatively charged particles in water solution. High percentages of capsaicin (70–90%) were incorporated in lignin NCs containing neem oil, confirming the versatility of this polymer to be loaded with hydrophobic compounds. DLS results showed that the obtained nanocapsules had an average size in the suitable range for drug delivery and concurred with higher resolution structural techniques (electron microscopy and SAXS) to establish that more than a single population of aggregates were present in solution. In particular, electron microscopy and SAXS revealed the presence of cylinders in the formulation N11, while in the N13 samples only spherical aggregates were detected. These anisotropic particles appear to play a major role in determining the observed better performance of the samples prepared at lower starting pH. Indeed, the test on *Eruca sativa* showed that NC11 exerted a greater protective effect than NC13, allowing constant growth of the aerial part.

In summary, this work provides a case study of how to use sustainable nanotechnology to counteract insecticide resistance, showing that the detailed design and characterization of formulations from natural sources is a viable strategy for sustainable plant protection. The nanovector design with plant-derived and safe materials lays

the groundwork for scaling up the procedure. In this kind of approach, LCA (life cycle assessment) could provide an added value guiding the right choice of production processes to reduce environmental impacts.[37]

Data availability

The data supporting the findings of the study will be made available as ESI† accompanying this article. The ESI† will include a detailed SAXS analysis that further substantiates the results reported in the main manuscript and raw data on insect experimental bioassays.

Author contributions

Sara Falsini: conceptualization, methodology, formal analysis, investigation, data curation, and writing – review and editing. Tommaso Nieri: methodology, formal analysis, investigation, and supervision. Alessio Papini: data curation, resources, and writing – review and editing. Maria Cristina Salvatici: investigation and writing – review and editing. Ali Abou-Hassan: investigation, data curation, and writing – review and editing. Cristina Gonnelli: conceptualization, methodology, resources, writing – review and editing, supervision, project administration, and funding acquisition. Sandra Ristori: conceptualization, methodology, resources, writing – review and editing, supervision, project administration, and funding acquisition. All authors read and approved the final manuscript.

Conflicts of interest

There are no conflicts to declare.

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References

1 FAO, IFAD, UNICEF, WFP and WHO, *The State of Food Security and Nutrition in the World 2019, Safeguarding against economic slowdowns and downturns*, Food and Agriculture Organization of the United Nations (FAO), Rome, 2019.

2 S. S. Ibrahim, Essential oil nanoformulations as a novel method for insect pest control in horticulture, *Horticultural crops*, 2019, ch. 10, DOI: **10.5772/intechopen.80747**.

3 G. V. Lowry, J. P. Giraldo, N. F. Steinmetz, A. Avellan, G. S. Demirer, K. D. Ristroph, G. J. Wang, C. O. Hendren, C. A. Alabi, A. Caparco, W. da Silva, I. González-Gamboa, K. D. Grieger, S.-J. Jeon, M. V. Khodakovskaya, H. Kohay, V. Kumar, R. Muthuramalingam, H. Poffenbarger, S. Santra, R. D. Tilton and J. C. White, Towards realizing nano-enabled precision delivery in plants, *Nat. Nanotechnol.*, 2024, **19**, 1255–1269, DOI: **10.1038/s41565-024-01667-5**.

4 D. Wang and J. C. White, Benefit of Nano-Enabled Agrochemicals, *Nat. Food*, 2022, **3**, 983–984, DOI: **10.1038/s43016-022-00665-x**.

5 M. Vurro, C. Miguel-Rojasb and A. Pérez-de-Luquec, Safe nanotechnologies for increasing the effectiveness of environmentally friendly natural agrochemicals, *Pest Manage. Sci.*, 2019, **75**, 2403–2412, DOI: **10.1002/ps.5348**.

6 S. Falsini, M. C. Rosi, E. Ravegnini, S. Schiff, C. Gonnelli, A. Papini, A. Adessi, S. Urciuoli and S. Ristori, Nanoformulations with exopolysaccharides from cyanobacteria: enhancing the efficacy of bioactive molecules in the Mediterranean fruit fly control, *Environ. Sci. Pollut. Res.*, 2023, **30**, 83760–83770, DOI: **10.1007/s11356-023-28180-x**.

7 E. V. R. Campos, J. L. de Oliveira, M. Pascoli, R. de Lima and L. F. Fraceto, Neem Oil and Crop Protection: From Now to the Future, *Front. Plant Sci.*, 2016, **7**, 1494, DOI: **10.3389/fpls.2016.01494**.

8 D. Pimentel and M. Burgess, Small amounts of pesticides reaching target insects, *Environ. Dev. Sustain.*, 2012, **14**, 1–2, DOI: **10.1007/s10668-011-9325-5**.

9 M. H. Sipponen, H. Lange, C. Crestini, A. Henn and M. Österberg, Lignin for Nano- and Microscaled Carrier Systems: Applications, Trends, and Challenges, *ChemSusChem*, 2019, **12**(10), 2039–2054, DOI: **10.1002/cssc.201900480**.

10 W. D. H. Schneider, A. J. P. Dillon and M. Camassola, Lignin nanoparticles enter the scene: A promising versatile green tool for multiple applications, *Biotechnol. Adv.*, 2021, **47**, 107685, DOI: **10.1016/j.biotechadv.2020.107685**.

11 M. H. Sipponen, A. Henn, P. Penttiläa and M. Österberg, Lignin-fatty acid hybrid nanocapsules for scalable thermal energy storage in phase-change materials, *Chem. Eng. J.*, 2020, **393**, 124711, DOI: **10.1016/j.cej.2020.124711**.

12 P. H. C. Lima, D. R. Antunes, M. M. L. Forini, M. S. Pontes, B. D. Mattos and R. Grillo, Recent Advances on Lignocellulosic-Based Nanopesticides for Agricultural Applications, *Front. Nanotechnol.*, 2021, **3**, 809329, DOI: **10.3389/fnano.2021.809329**.

13 J. Fischer, S. J. Beckers, D. Yiamsawas, E. Thines, K. Landfester and F. R. Wurm, Targeted Drug Delivery in Plants: Enzyme-Responsive Lignin Nanocarriers for the Curative Treatment of the Worldwide Grapevine Trunk Disease Esca, *Adv. Sci.*, 2019, **6**, 1802315, DOI: **10.1002/advs.201802315**.

14 S. Peil, S. J. Beckers, J. Fischer and F. R. Wurma, Biodegradable, lignin-based encapsulation enables delivery of Trichoderma reesei with programmed enzymatic release against grapevine trunk diseases, *Mater. Today Bio*, 2020, **7**, 100061, DOI: **10.1016/j.mtbio.2020.100061**.

15 S. Falsini, I. Clemente, A. Papini, C. Tani, S. Schiff, M. C. Salvatici, R. Petruccelli, C. Benelli, C. Giordano, C. Gonnelli and S. Ristori, When Sustainable Nanochemistry Meets Agriculture: Lignin Nanocapsules for Bioactive Compound Delivery to Plantlets, *ACS Sustainable Chem. Eng.*, 2019, **7**(24), 19935–19942, DOI: **10.1021/acssuschemeng.9b05462**.

16 S. Falsini, T. Nieri, A. Paolini, S. Schiff, A. Papini, L. Mugnai, C. Gonnelli and S. Ristori, Tannins-lignin mixed nanoformulations for improving the potential of neem oil as fungicide agent, *Environ. Sci. Pollut. Res.*, 2023, **30**, 39131–39141, DOI: **10.1007/s11356-022-24991-6**.

17 A. Barhoum, J. Jeevanandam, A. Rastogi, P. Samyn, Y. Boluk, A. Dufresne, M. K. Danquah and M. Bechelany, Plant celluloses, hemicelluloses, lignins, and volatile oils for the synthesis of nanoparticles and nanostructured materials, *Nanoscale*, 2020, **12**, 22845–22890, DOI: **10.1039/D0NR04795C**.

18 F. Zikeli, V. Vinciguerra, S. Sennato, G. Scarascia Mugnozza and M. Romagnoli, Preparation of Lignin Nanoparticles with Entrapped Essential Oil as a Bio-Based Biocide Delivery System 2020, *ACS Omega*, 2019, **5**(1), 358–368, DOI: **10.1021/acsomega.9b02793**.

19 A. E. Santo Pereira, J. L. de Oliveira, S. M. Savassa, C. B. Rogério, G. A. de Medeiros and L. F. Fraceto, Lignin nanoparticles: New insights for a sustainable agriculture, 2022, *J. Cleaner Prod.*, 2022, **345**, 131145, DOI: **10.1016/j.jclepro.2022.131145**.

20 S. Falsini, T. Nieri, S. Schiff, A. Papini, M. C. Salvatici, G. Carella, L. Mugnai, C. Gonnelli and S. Ristori, Enhancing the Efficacy of Natural Repellents Against Grapevine Pathogens by Tannins-Lignin-Mixed Nanovectors, *Bionanoscience*, 2024, **14**, 474–484, DOI: **10.1007/s12668-023-01244-5**.

21 M. B. Isman, Neem and other Botanical insecticides: Barriers to commercialization, *Phytoparasitica*, 1997, **25**, 339–344, DOI: **10.1007/BF02981099**.

22 R. Kumar, S. Mehta and S. R. Pathak, Bioactive constituents of neem, *Synthesis of medicinal agents from plants*, 2018, ch. 4, pp. 75–103, DOI: **10.1016/B978-0-08-102071-5.00004-0**.

23 F. M. Shah, M. Razaq, A. Ali, P. Han and J., Chen Comparative role of neem seed extract, moringa leaf extract and imidacloprid in the management of wheat aphids in relation to yield losses in Pakistan, *PLoS One*, 2017, **12**(9), 1–24, DOI: **10.1371/journal.pone.0184639**.

24 N. E. S. Lale, Oviposition-deterrent and repellent effects of products from dry chilli pepper fruits, Capsicum species on Callosobruchus maculatus, *Postharvest Biol. Technol.*, 1992, **1**(4), 343–348, DOI: **10.1016/0925-5214(92)90036-O**.

25 Y. Li, P. Bai, L. Wei, R. Kang, L. Chen, M. Zhang, E. K. Tan and W. Liu, Capsaicin Functions as Drosophila Ovipositional Repellent and Causes Intestinal Dysplasia, *Sci. Rep.*, 2020, **10**, 9963, DOI: **10.1038/s41598-020-66900-2**.

26 N. S. Talekar and A. M. Shelton, Biology, ecology, and management of the diamondback moth, *Annu. Rev. Entomol.*, 1993, **38**(1), 275–301, DOI: **10.1146/annurev.en.38.010193.001423**.

27 A. A. Sial and A. J. Brunner, Lethal and sublethal effects of an insect growth regulator, pyriproxyfen, on obliquebanded leafroller (Lepidoptera: Tortricidae), *J. Econ. Entomol.*, 2010, **103**, 340–347, DOI: **10.1603/ec09295**.

28 C. A. Schneider, W. S. Rasband and K. W. Eliceiri, NIH Image to ImageJ: 25 years of image analysis, *Nat. Methods*, 2012, **9**(7), 671–675, DOI: **10.1038/nmeth.2089**.

29 P. Tomani, The lignoboost process, *Cellul. Chem. Technol.*, 2010, **44**, 53.

30 T. Sewring, J. Durruty, L. Schneider, H. Schneider, T. Mattsson and H. Theliander, Acid precipitation of kraft lignin from aqueous solutions: the influence of pH, temperature, and xylan, *J. Wood Chem. Technol.*, 2019, **39**(1), 1–13.

31 S. K. Tulashie, F. Adjei, J. Abraham and E. Addo, Potential of neem extracts as natural insecticide against fall armyworm (*Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), *Case Stud. Chem. Environ. Eng.*, 2021, **4**, 100130, DOI: **10.1016/j.cscee.2021.100130**.

32 T. O. Machado, S. J. Beckers and J. Fischer, *et al.*, Bio-Based Lignin Nanocarriers Loaded with Fungicides as a Versatile Platform for Drug Delivery in Plants, *Biomacromolecules*, 2020, **21**(7), 2755–2763, DOI: **10.1021/acs.biomac.0c00487**.

33 R. P. Singh, R. Handa and G. Manchanda, Nanoparticles in sustainable agriculture: an emerging opportunity, *J. Controlled Release*, 2021, **329**, 1234–1248, DOI: **10.1016/j.jconrel.2020.10.051**.

34 S. Salentinig and M. Schubert, Softwood lignin self-assembly for nanomaterial design, *Biomacromolecules*, 2017, **18**, 2649–2653, DOI: **10.1021/acs.biomac.7b00822**.

35 M. B. Agustin, P. A. Penttilä, M. Lahtinen and K. S. Mikkonen, Rapid and Direct Preparation of Lignin Nanoparticles from Alkaline Pulping Liquor by Mild Ultrasonication, *ACS Sustainable Chem. Eng.*, 2019, **7**, 19925–19934, DOI: **10.1021/acssuschemeng.9b05445**.

36 M. Weiss, J. Fan and M. Claudel, *et al.*, Density of surface charge is a more predictive factor of the toxicity of cationic carbon nanoparticles than zeta potential, *J. Nanobiotechnol.*, 2021, **19**, 5, DOI: **10.1186/s12951-020-00747-7**.

37 S. Falsini, U. Bardi, A. Abou-Hassan and S. Ristori, Sustainable strategies for large-scale nanotechnology manufacturing in the biomedical field, *Green Chem.*, 2018, **20**, 3897, DOI: **10.1039/c8gc01248b**.