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Efficacy of lignin from grape stalks in the sustainable management of the root-knot nematode *Meloidogyne luci*

Ana C. Cassoni [a], Carla Maleita [b,c,d], Isabel Abrantes [d], Manuela Pintado [a], Marta Vasconcelos [a,*]

[a] Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, Porto, 4169-005, Portugal
[b] Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Oeiras, 2780-159, Portugal
[c] CERES - Chemical Engineering and Renewable Resources for Sustainability, Department of Chemical Engineering, University of Coimbra, Coimbra, Coimbra, 3030-790, Portugal
[d] Centre for Functional Ecology: Science for People & Planet (CFE), Associate Laboratory TERRA, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, Coimbra, 3000-456, Portugal

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ABSTRACT

Root-knot nematodes (RKN, *Meloidogyne* spp.) cause substantial agricultural losses worldwide, and sustainable alternatives to synthetic nematicides are needed. This study evaluated the potential bionematicidal effects of two formulations based on micronized and nanoformulated lignin extracted from grape stalks via alkaline extraction and deep eutectic solvent (DES) methods against the RKN *M. luci*. *In vitro* assays revealed that micronized lignin achieved moderate second-stage juveniles (J2) mortality (maximum 66.5% at 30 mg/mL). Lignin nanoparticles (LigNPs) markedly improved efficiency, causing 68.6% J2 mortality at 0.250 mg/mL (representing a 99% reduction in concentration). Alkaline LigNPs demonstrated high J2 hatching inhibition (87.8% at 0.468 mg/mL), while DES LigNPs induced 44.6% inhibition at 0.250 mg/mL. Soil assays, performed to confirm *in vitro* results, revealed differential performance: J2 mortality decreased substantially (31.8% for DES at 0.5 mg/mL, negligible for alkaline at 0.94 mg/mL), likely due to adsorption in soil and reduced bioavailability. However, DES LigNPs maintained significant J2 hatching inhibition in soil (66.9% at 0.5 mg/mL), suggesting their potential for preventive applications. Hypothetical nematicidal mechanisms are discussed. Although requiring higher concentrations than metallic nanoparticles, lignin offers advantages including biodegradability, agricultural waste valorisation, and environmental safety. This first demonstration of LigNPs as bionematicides establishes a foundation for developing new sustainable RKN management strategies based on agricultural byproducts.

1. Introduction

Root-knot nematodes (RKN, *Meloidogyne* spp.) are among the most economically devastating plant-parasitic nematodes worldwide, affecting over 3000 plant species [1–3] and causing annual crop losses estimated at 100 to 358 USD billion globally [4,5]. Root-knot nematodes are obligatory sedentary endoparasites that establish feeding sites within plant host roots, inducing characteristic galls that disrupt water and nutrient transport, compromising plant growth and yield and, ultimately, plant survival [1,3,6].

Current nematode control strategies are mostly based on synthetic nematicides, which pose environmental and human health risks, leading to increasing regulatory restrictions and market withdrawals [6]. Recent reviews show an increasing interest in developing sustainable and environmentally friendly alternative strategies. However, biological control approaches, including bacteria and fungi, plant essential oils, and cultural practices, have shown promise, but many challenges must be overcome [4,6–8].

Plant resistance mechanisms against RKN often involve enhanced lignification of root tissues, creating physical and chemical barriers to nematode penetration and the establishment of feeding sites [9,10]. Lignin, a complex phenolic polymer comprising 15-30% of lignocellulosic biomass, plays an important role in plant defence mechanism [11,12]. Studies have demonstrated that resistant plants respond to

* Corresponding author.
E-mail address: mvasconcelos@ucp.pt (M. Vasconcelos).

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nematode penetration by upregulating lignin biosynthesis genes, resulting in localized lignin deposition [10,13].

Despite the extensive available literature on lignification as a defence reaction, direct application of extracted lignin as a nematicidal agent remains unexplored. Current research has focused predominantly on enhancing endogenous plant lignification through chemical elicitors or genetic modification [3,8,10], rather than evaluating lignin itself as a biopesticide. This represents a significant knowledge gap, particularly given that lignocellulosic biomass is the most abundant biomass resource on Earth and lignin is still underutilized [14,15].

This study represents the first comprehensive evaluation of the bio-nematicidal potential of extracted lignin from grape (*Vitis vinifera*) stalks against the RKN *M. luci*. Our specific objectives were to (1) compare the nematicidal efficiency of lignin extracted from grape stalks using alkaline and deep eutectic solvent (DES) methods, and (2) evaluate the impact of particle size reduction through micronization and nano-formulation on biological activity, through *in vitro* second-stage juvenile (J2) mortality and hatching inhibition assays; and (3) determine the persistence of nematicidal activity in soil environments. This research addresses a critical gap in sustainable nematode management while advancing the valorisation of agricultural waste streams.

2. Materials and methods

High purity reagents used in this work, sodium hydroxide (NaOH), sulphuric acid (H_2SO_4), lactic acid (LA), choline chloride (CC), and Tween 80, were purchased from Merck KGaA (Darmstadt, Germany).

2.1. Lignin extraction and processing

Grape stalks from cv. Vinhão were kindly provided by Quinta de Mascate (Vila Verde, Portugal). Biomass was dried, milled and stored at room temperature during the experiments.

Lignin was isolated from grape stalks using two distinct extraction methods previously optimized by Cassoni et al. [16]. The alkaline method consisted of lignin treatment with 6% NaOH solution at 80 °C for 1 h, whereas in the DES method a lactic acid:choline chloride mixture (5:1 M ratio) at 120 °C for 5 h was applied. Subsequently, the dried lignin extracts underwent micronization using a Planetary Ball Mill PM 100 (Retsch GmbH, Germany), with alkaline lignin (AkL) processed for 5 min and DES lignin (DESL) for 20 min to achieve homogeneous micronized lignin powders. The size of lignin particles was measured in a MasterSizer Hydro 3000 equipped with a Hydro EV dispersion unit (Malvern Instruments, Worcestershire, UK) using an obscuration range of 5-10%. The sample was dispersed in water and ultrasonicated externally for 5 min. Prior to analysis, the sample was submitted to 1 min of ultrasounds and stirred at 2500 rpm. In the estimation of the particle size distribution, it was considered non-spherical particles with a refractive index of 1.64 and absorption index of 0.01.

Lignin nanoparticles (LigNPs) were synthesized through ultrasonication following the protocol established by Cassoni et al. [17]. Briefly, micronized lignin powders were suspended in 0.05% (w/w) Tween 80 solution and processed using an Ultrasonic processor Q700X (Qsonica, Newtown, CT, USA) equipped with a 10 mm probe. Throughout sonication, samples were maintained in an ice bath under continuous agitation to prevent thermal degradation. The resulting nanoparticle suspensions were subsequently concentrated by two-fold through controlled evaporation at 45 °C. LigNPs samples were analysed using DLS with a Zetasizer Nano ZSP (Malvern Panalytical Ltd., Malvern, UK), which assessed particle size, polydispersity index (PDI), and zeta potential (ZP).

2.2. Nematode isolate - maintenance and inoculum

The RKN *M. luci* isolate was selected from the RKN NEMATO-lab collection (Laboratory of Nematology, Department of Life Sciences, University of Coimbra). The *M. luci* isolate was originally obtained from potato roots in Cantanhede, Coimbra, Portugal [18] by inoculation of a single egg mass and was maintained on tomato cv. Coração-de-Boi, in pots containing sterilized sandy loam soil and sand (1:1 v/v), at 25 ± 2 °C in a growth chamber.

To obtain J2, infected tomato plants were uprooted and roots thoroughly washed to remove adhering soil. Then, roots were sectioned into small fragments and examined under a stereomicroscope for egg mass collection. Egg masses were handpicked and transferred to a fine-mesh sieve suspended over a collection dish containing sterile tap water. The egg masses were maintained at 22 °C, and J2 hatched during the first 24 h were discarded. Subsequently, freshly emerged J2 were collected at 24 h intervals for up to 5 consecutive days and were stored at 4 °C until experimental use.

Meloidogyne luci eggs were extracted from egg masses collected from infected tomato roots using a 0.52% NaOCl solution [19].

2.3. Nematicidal activity assessment

2.3.1. Micronized lignin

Nematicidal activity of micronized lignin was evaluated through J2 mortality assay. Prior to experimental setup, J2 were maintained under constant agitation (400 rpm). Number of nematodes was determined through triplicate counts of 500 µL aliquots using a counting chamber under stereomicroscopic observation. The suspension volume was adjusted to achieve a final concentration of 50-60 J2 per 500 µL.

Bioassays were conducted with six replicates per treatment. Based on previous studies [20,21], which showed that high concentrations of solid lignin are needed to inhibit certain bacteria, the micronized lignin concentration used in this study was 30 mg/mL. Micronized lignin samples (both AkL and DESL) were placed in individual wells of a 24-well plate, followed by the addition of 500 µL of standardized J2 suspension. Sterile tap water was included as control. Plates were covered to maintain darkness and incubated at 22 °C under continuous agitation for 24 or 48 h. Given the destructive nature of the assessment, separate plate sets were prepared for each time point.

Following incubation, nematode viability/mortality was assessed using the modified generalist Tray Method [22]. Mini sieves were constructed using PVC tubes of 2 cm Ø fitted with 200 µm mesh fabric secured with elastic bands and placed over small Petri dishes. The lignin-nematode suspensions were carefully transferred to the mini-sieves, and each well was rinsed twice with 1 mL of sterile water. Additional water (approximately 2 mL) was added to maintain nematode submersion. The assemblies were incubated in darkness at room temperature for another 24 h to allow motile nematodes to migrate through the mesh.

After migration, the sieves were removed, and the collected filtrate, containing viable nematodes, was transferred to counting plates. Petri dishes were rinsed with water to ensure complete nematode recovery, counting the total mobile J2 under a stereoscope. Nematodes not showing movements were touched with a bristle and considered dead if they failed to react.

2.3.2. Lignin nanoparticles

The J2 mortality and hatching inhibition were evaluated for the LigNPs, *in vitro* and *in soil* assays. LigNPs maximum achieved concentration was 0.94 (±0.05) mg/mL for AkL and 0.50 (±0.04) mg/mL for DESL, since lignin load was optimized for the ultrasonication process and higher lignin concentrations produced larger LigNPs.

2.3.2.1. In vitro assays. Meloidogyne luci J2 mortality assay was performed as previously described for micronized lignin, with some modifications. Bioassays were conducted in 24-well plates with 5 replicates per treatment, and sterile tap water was included as control. Each well received 500 µL of standardized J2 suspension, followed by an equal

volume of LigNP suspension, resulting in final concentrations of nanoparticles of 0.468 and 0.250 mg/mL for AkL and DESL, respectively. Nematode viability/mortality was assessed 24 and 48 h after incubation. Since the LigNPs suspension was dark, agitation was halted, and after 30 min of rest, most of the liquid was carefully removed. Then, 1 mL of water was added to facilitate nematode counting. When immobility exceeded 50%, incubation was extended by an additional 24 h. Following this recovery period, immobility indicated nematicidal activity, while restored mobility suggested a nematostatic effect.

The effect of LigNPs on J2 hatching inhibition was also evaluated using the same methodology described for mortality assay, with some modifications. Bioassays were conducted in 96-well plates with 5 replicates per treatment. The number of eggs was adjusted to approximately 50 eggs/50 µL, and each well received 50 µL of egg suspension. Before adding an equal volume of lignin nanoparticle suspension or sterile tap water (control), the number of hatched J2 and eggs was recorded. Plates were covered to maintain darkness and incubated at 22-24 °C under continuous agitation. Hatching was assessed 10 days post-treatment by stereomicroscopic observation. Due to the dark coloration of LigNPs suspensions, which limit direct visualization, a portion of each suspension was withdrawn and diluted with water to enable the accurate evaluation of eggs and emerged J2.

2.3.2.2. Soil-based assays. The efficiency of LigNPs in managing RKN in a soil matrix was evaluated using sandy loam soil. For control treatments, soil moisture was adjusted to 40% water holding capacity (WHC). For nanoparticle treatments, soil moisture was adjusted to 40% WHC using the respective lignin nanoparticle suspensions (0.94 and 0.50 mg/mL of AkL and DESL, respectively) instead of water. Approximately 60 g of moistened soil (corresponding to ~50 g dry weight) was distributed into individual flasks (5 flasks/treatment).

For J2 mortality and hatching inhibition assays, each replicate/treatment was inoculated with 1 mL of nematode suspension containing approximately 200 J2 or 1250 eggs, respectively. Flasks were incubated in darkness at 22-24 °C for 5 days (J2 mortality assay) or 7 days (J2 hatching inhibition assay). Following incubation, RKN J2 were extracted from soil using the modified generalist Tray Method [22], for 24 h to allow viable nematodes to migrate to the water. Nematicidal efficacy was calculated based on the reduction of nematode population recovered compared to water-treated control.

2.4. Data analysis

Data on effects of AkL and DESL on J2 mortality and hatching in *in vitro* and in soil assays were corrected by Abbott formula [23]:

$$Corrected\ mortality = \left(\frac{J2\ in\ treatment - J2\ in\ control}{J2\ in\ control}\right) x\ 100$$

Statistical analyses were performed using Student's t-tests or One-way ANOVA, followed by Tukey's HSD post-hoc test for multiple comparisons in IBM SPSS Statistics (version 26). Results are presented as mean ± standard deviation (SD). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Nematicidal activity assessment

3.1.1. Micronized lignin

Evaluation of the effect of micronized lignin revealed a moderate activity against *M. luci* J2 at 30 mg/mL (Fig. 1). Due to the high variability, no significant difference was found ($p > 0.05$) between both time points or lignin types. The *M. luci* J2 mortality in the water control after 24 h and 48 h of exposure was 28.36 ± 15.88% and 23.27 ± 17.88%, respectively.

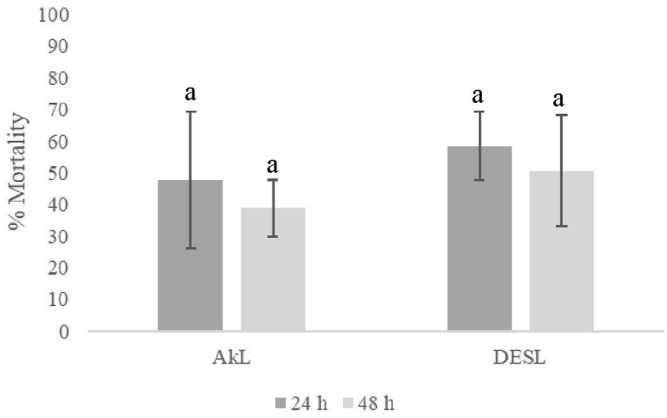

Fig. 1. – Corrected mortality (%) of *Meloidogyne luci* second-stage juveniles (J2) following exposure to micronized alkaline lignin (AkL) and deep eutectic solvent lignin (DESL) at 30 mg/mL, by 24 and 48 h. Data were corrected with reference to water and are means ± SD of five replicates. Bars sharing a common lower-case letter are not significantly different ($p > 0.05$) according to Student's t-test.

3.1.2. Lignin nanoparticles

3.1.2.1. In vitro. Lignin nanoparticles improved nematicidal efficiency against *M. luci* J2 compared to micronized lignin (Fig. 2). After 24 h of exposure, AkL nanoparticles (0.94 ± 0.05 mg/mL) showed a mortality significantly higher than its control, while DESL nanoparticles (0.50 ± 0.05 mg/mL) further increased this effect. Extended exposure to 48 h further enhanced nematicidal activity, with significant differences ($p < 0.05$) between time points and LigNPs.

Regarding J2 hatching inhibition, *in vitro* evaluation revealed strong inhibitory activity, particularly for AkL formulations (Fig. 3).

3.1.2.2. Soil assays. Soil-based assays revealed that DESL nanoparticles retain substantial nematicidal activity despite the complex soil environment (Fig. 4).

Regarding J2 mortality, no effects were observed with AkL LigNPs, while DESL caused a significantly higher mortality. Regarding J2 hatching inhibition, AkL nanoparticles J2 hatching inhibition was also not different from control, while DESL nanoparticles achieved a significantly higher hatching inhibition.

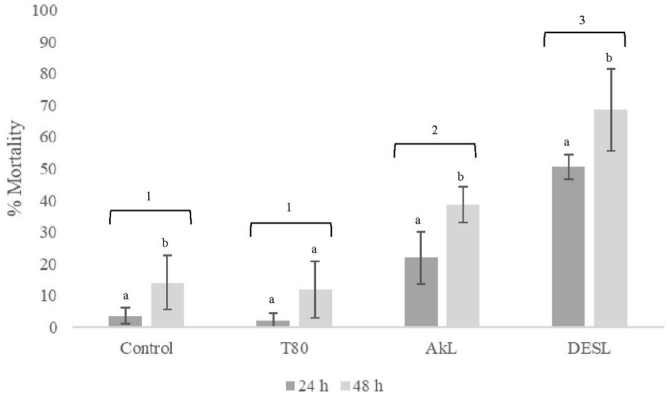

Fig. 2. Mortality (%) of *Meloidogyne luci* second-stage juveniles (J2) following exposure to lignin nanoparticles derived from alkaline (AkL) and deep eutectic solvent (DESL) extraction methods, at 0.94 ± 0.05 and 0.50 ± 0.05 mg/mL, respectively. Controls include water (Control) and 0.05 % Tween 80 (T80) used as the nanoparticle dispersant. Data are means ± SD of five replicates. Bars and groups sharing a common lower-case letter or number, respectively, are not significantly different ($p > 0.05$), according to One-way ANOVA, followed by Tukey's HSD post-hoc test for multiple comparisons.

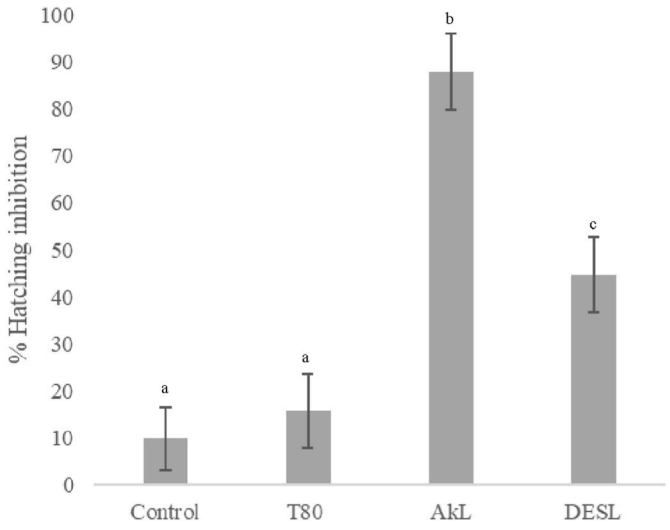

Fig. 3. – *Meloidogyne luci* second-stage juveniles hatching inhibition following exposure to lignin nanoparticles derived from alkaline (AkL) and deep eutectic solvent (DESL) extraction methods, at 0.94 ± 0.05 and 0.50 ± 0.05 mg/mL, respectively. Controls include water (Control) and 0.05 % Tween 80 (T80) used as the nanoparticle dispersant. Data are means ± SD of five replicates. Bars sharing a common lower-case letter are not significantly different ($p > 0.05$), according to One-way ANOVA, followed by Tukey's HSD post-hoc test for multiple comparisons.

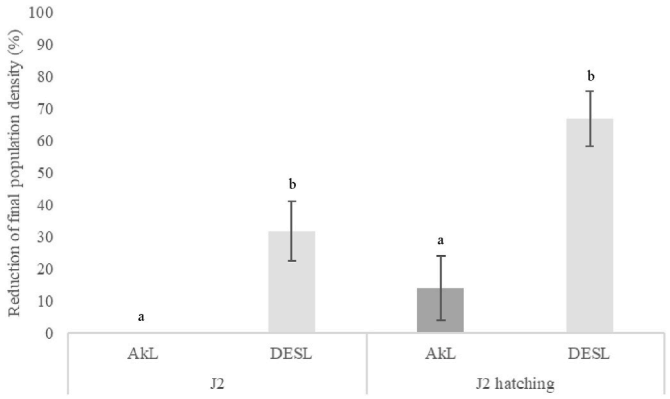

Fig. 4. – Corrected final population density (%) reduction of *Meloidogyne luci* second-stage juveniles (J2) after exposure to alkaline (AkL) and deep eutectic solvent (DESL) lignin nanoparticles during 5 days (J2 mortality) and 7 days (J2 hatching inhibition), at 0.94 ± 0.05 and 0.50 ± 0.05 mg/mL, respectively. Data were corrected with reference to water and are means ± SD of five replicates. Bars sharing a common lower-case letter are not significantly different ($p > 0.05$), according to Student's t-test. Statistical analysis was performed in separate for J2 and J2 hatching.

4. Discussion

Besides the inherent variability of biological assays due to factors such as physiological state and population heterogeneity [24], the observed variability could also be attributed to factors related to the micronized lignin. First, the heterogeneous particle size distribution could create inconsistent nematode-particle interactions within test wells.

The mechanisms of lignin assumption by the nematodes are not known. The heterogeneity is particularly relevant given that the stylet aperture of J2 is very small (1-2 μm) [25,26], preventing particles larger than this threshold from ingestion. The high concentration required for moderate efficacy (30 mg/mL) suggests that only a small fraction of particles (those below the critical size threshold) may have contributed to the observed nematicidal activity through possible direct ingestion.

The cuticle, a multilayered structure comprising collagen-like proteins and lipids, plays a crucial role in the nematode defence mechanism [26]. This protective layer likely prevents the penetration of lignin particles through the body wall, thus, nematicidal effects may be potentially achieved through ingestion and/or interaction between lignin particles and the components of the cuticle.

Following ingestion, phenolic compounds, abundant in the lignin structure, could significantly contribute to the nematicidal effect through multiple mechanisms [4,5,7,27–29]. Phenolic compounds with hydroxyl groups induce oxidative stress by producing reactive oxygen species (ROS), which lead to oxidative damage and activation of apoptotic pathways [4,30]. Aissani et al. [27] reported that redox-active phenols disrupt critical physiological processes in nematodes, including inhibition of vacuolar-type H + -ATPase, membrane damage through lipid peroxidation, and depletion of antioxidant enzyme systems. This multifaceted oxidative stress can impair the nematode defence mechanisms, ultimately leading to death.

As expected, the nematicidal effect of micronized lignin is inferior compared to other sustainable alternatives reported in the literature [31–35] evaluating soluble extracts that achieve activity at significantly lower concentrations. Solid micronized lignin may face limitations in bioavailability, as the heterogeneous particle size and limited solubility restrict nematode access to the bioactive components, requiring higher concentrations to achieve comparable effects.

The enhanced performance of LigNPs corroborates the hypothesis that particle size reduction, below the nematode stylet aperture threshold, might improve nematicidal activity. While maximum mortality rates were comparable between micronized lignin and nanoparticles (58.4% and 68.6% respectively), the concentration required differed, with LigNPs achieving similar efficacy with approximately 99% reduction in required concentration (from 30 mg/mL to 0.5 mg/mL). The higher surface area could increase bioactivity and phenolic compound release, which contribute to higher efficiency. The enhanced bioavailability and higher surface reactivity of LigNPs may enable direct interactions with cuticle proteins or cellular proteins and nucleic acids, causing broader physiological disruption than achieved with larger particles. This could increase oxidative stress and disrupt multiple cellular mechanisms, including alterations in cell membrane permeability and damage, DNA damage and oxidation, and cell-cycle disruption [34,36].

Comparing AkL and DESL, DESL demonstrated superior nematicidal performance with significant differences ($p < 0.05$). This enhanced efficiency may be due to its higher purity (89.7%) and antioxidant activity [16], which may increase oxidative stress. Moreover, DESL nanoparticles have a more uniform, spherical morphology than AkL [16] potentially enabling more consistent uptake and improved dispersion. To the best of our knowledge, this is the first study to evaluate the nematicidal activity of LigNPs against RKN. Recent research has focused on the green synthesis of metallic nanoparticles, particularly silver, as sustainable nematicidal agents against RKN. Our lignin nanoparticle results (68.6% J2 mortality at 0.250 mg/mL) fall within the range reported for metallic nanoformulations. Comparable mortality rates have been achieved with various metal-based systems: Nazir et al. [37] reported 40% *M. incognita* J2 mortality using 100 mg/mL of silver nanoparticles, while Khan et al. [38] synthesized copper oxide nanoparticles, achieving 84.4% mortality at 0.200 mg/mL. Similarly, Heflish et al. [39] observed 53.3% mortality of *M. incognita* after 48 h of exposure to 0.100 mg/mL silver nanoparticles.

However, certain studies on metallic nanoparticles have demonstrated superior efficacy compared to LigNPs. Cromwell et al. [36] achieved 99% *M. incognita* J2 reduction with 0.150 mg/mL silver nanoparticles, while Arumugam et al. [40] reported complete mortality at 0.200 mg/mL. Remarkably, Rani et al. [41] demonstrated high efficiency against *M. incognita* using silver nanoparticle concentrations as low as 0.01 mg/mL. While metallic nanoparticles may offer higher

effectiveness at lower concentrations, LigNPs present distinct advantages such as biodegradability and lower environmental persistence [42,43].

The mechanism of hatching inhibition is likely complex and may involve multiple targets within the egg structure, making it difficult to elucidate the mode of action.

The J2 hatching inhibition efficiency of LigNPs is comparable to that reported in other studies. Arumugam et al. [40] reported 24% J2 hatching inhibition using 0.2 mg/mL silver nanoparticles, which is substantially lower than our results for both AkL (87.8%) and DESL (44.6%) formulations. Similarly, Nazir et al. [37] required a considerably higher concentration of silver nanoparticles (100 mg/mL) to achieve only 40% *M. incognita* J2 hatching inhibition. Khan et al. [38] obtained 80% inhibition using 0.200 mg/mL copper oxide nanoparticles, which is consistent with our AkL nanoparticle performance.

However, as observed with J2 mortality, several studies have demonstrated superior nematode hatching inhibition at lower concentrations. Rani et al. [41] achieved pronounced J2 hatching inhibition with silver nanoparticles at 0.01 mg/mL, while Kalaiselvi et al. [44] reported almost total inhibition at an extremely low concentration of 0.001 mg/mL silver nanoparticles.

The differences in efficiency observed between soil and *in vitro* conditions highlight the potential influence of the soil matrix on nanoparticle bioavailability and nematicidal activity. Soil-related factors such as pH, organic matter content, degradation of active ingredients, chemical composition, biological activity, and temperature can influence the effectiveness of treatments [6].

The reduced J2 mortality in soil compared to *in vitro* conditions (0% vs. 38.7% for AkL; 31.8% vs. 68.6% for DESL) reflects the challenges of delivering nematicides in soil. The low concentration of LigNPs combined with their dispersion through the soil may hinder direct nematode-particle contact. Moreover, the soil may neutralize the effect of extraction-derived residues, eliminating potential synergistic effects that could contribute to nematicidal activity [6]. These factors may also explain the low hatching inhibition of AkL nanoparticle in soil.

Remarkably, DESL nanoparticles maintained high J2 hatching inhibition in soil (66.8 $\pm$ 8.5%). Hence, this assay was repeated for results validation. The replicate assay confirmed this efficiency with 47.6% nematode hatching inhibition, showing no significant differences from the initial trial ($p > 0.05$). However, under natural conditions, RKN eggs are enclosed within the egg mass, which has a protective gelatinous matrix, providing a degree of physical and biochemical defence [26,45]. In the present study, eggs were isolated from the egg masses prior to exposure, likely increasing their exposure to LigNPs.

Our soil assay results show comparable efficiency to those of Khan et al. [38], who reported a 67% J2 reduction using 0.200 mg/mL copper oxide nanoparticles in soil, representing only a slight decrease from their *in vitro* performance, similar to our findings. However, several studies have achieved superior nematicidal activity. Silver nanoparticle root-dip treatments achieved complete J2 mortality and hatching inhibition at concentrations as low as 0.001 mg/mL [44]. Similarly, Daramola et al. [46] observed a significant reduction in the number of egg masses and J2 population of *M. javanica* using only 0.03 mg/mL silver nanoparticles.

While some researchers document efficiency losses in soil environments, the results generally exceed the performance of our LigNPs. Cromwell et al. [36] noted reduced efficiency in soil compared to *in vitro* conditions yet still achieved a 92% J2 reduction at 0.150 mg/mL silver nanoparticles. Arumugam et al. [40] demonstrated substantial *in vivo* success with 93.1% reduction in the number of egg masses and a significant *M. incognita* population decrease using 0.100 mg/mL silver nanoparticles.

For a comprehensive evaluation of the efficiency of LigNPs in RKN management, soil studies involving the host plant will be essential, which will also enable the assessment of any positive effects on the plants. Furthermore, direct application to the rhizosphere with repeated treatments at short intervals may help overcome some difficulties related to the soil physicochemical characteristics by maintaining localized high concentrations. However, to achieve efficiency comparable to metallic nanoparticles, substantially higher lignin concentrations would be required.

5. Conclusions

Lignin extracted from agricultural byproducts can be an effective bionematicide against nematodes of the genus Meloidogyne, with efficiency dependent on particle size and extraction method. Micronized lignin exhibited limited nematicidal activity, but LigNPs overcame this limitation, achieving high mortality at low concentrations. This confirms that particle size is an important factor in the nematicidal activity of lignin.

The extraction method also significantly influenced nematicidal activity, with DESL nanoparticles more effective at inducing J2 mortality and AkL nanoparticles more effective at inhibiting hatching. Alkaline treatment yielded lower-molecular-weight fragments, while the DES method provided higher lignin purity and antioxidant activity.

Soil application revealed the challenges of maintaining nematicidal efficacy in complex environmental matrices. The persistence of hatching inhibition indicates that LigNPs could be valuable in integrated management strategies aiming at population suppression.

Although literature on metallic nanoparticles indicates higher efficiency at lower concentrations, LigNPs may offer distinct advantages, including biodegradability, valorisation of agricultural waste, minimal environmental persistence, no metal accumulation in soils, and possible compatibility with organic farming systems.

CRediT authorship contribution statement

Ana C. Cassoni: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Carla Maleita:** Conceptualization, Investigation, Methodology, Resources, Supervision, Writing – review & editing. **Isabel Abrantes:** Resources, Supervision. **Manuela Pintado:** Supervision, Validation, Writing – review & editing. **Marta Vasconcelos:** Conceptualization, Funding acquisition, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

References

[1] P. Abad, B. Favery, M.N. Rosso, P. Castagnone-Sereno, Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction, Mol. Plant Pathol. 4 (4) (2003) 217–224, https://doi.org/10.1046/j.1364-3703.2003.00170.x.

[2] T. Ali, M. Mubeen, Y. Jamil, U. Ahmad, H. Ali Khan, Y. Baber, P. Farazia Hassan, H. Muhammad Usman, M. Aamir Sohail, A. Abbas, S. Iqbal, F. Hassan, An overview of root-knot nematodes and their management, Journal of Entomology and Zoology Studies 9 (1) (2021) 35–40. http://www.entomoljournal.com.

[3] N.H.A.A. Ralmi, M.M. Khandaker, N. Mat, Occurrence and control of root knot nematode in crops: a review, Aust. J. Crop. Sci. 10 (12) (2016) 1649–1654, https://doi.org/10.21475/ajcs.2016.10.12.p7444.

[4] L. Azlay, M.E.M. El Boukhari, E.H. Mayad, M. Barakate, Biological management of root-knot nematodes (*Meloidogyne* spp.): a review, Org. Agric. 13 (1) (2023) 99–117, https://doi.org/10.1007/s13165-022-00417-y.

[5] H. Ibrahim, V.A. Nchiozem-Ngnitedem, L.M. Dandurand, I. Popova, Naturally-occurring nematicides of plant origin: two decades of novel chemistries, Pest Manag. Sci. 81 (2) (2025) 540–571, https://doi.org/10.1002/ps.8504.

[6] F. Forghani, A. Hajihassani, Recent advances in the development of environmentally benign treatments to control root-knot nematodes, Front. Plant Sci. 11 (2020) 1–13, https://doi.org/10.3389/fpls.2020.01125.

[7] N.G. Ntalli, P. Caboni, Botanical nematicides: a review, J. Agric. Food Chem. 60 (40) (2012) 9929–9940, https://doi.org/10.1021/jf303107j.

[8] A. Seid, C. Fininsa, T. Mekete, W. Decraemer, W.M.L. Wesemael, Tomato (*Solanum lycopersicum*) and root-knot nematodes (*Meloidogyne* spp.)-a century-old battle, Nematology 17 (9) (2015) 995–1009, https://doi.org/10.1163/15685411-00002935.

[9] F. Shu, B. Jiang, Y. Yuan, M. Li, W. Wu, Y. Jin, H. Xiao, Biological activities and emerging roles of lignin and lignin-based products - a Review, Biomacromolecules 22 (12) (2021) 4905–4918, https://doi.org/10.1021/acs.biomac.1c00805.

[10] P. Veronico, C. Paciolla, F. Pomar, S. De Leonardis, A. García-Ulloa, M.T. Melillo, Changes in lignin biosynthesis and monomer composition in response to benzothiadiazole and root-knot nematode *Meloidogyne incognita* infection in tomato, J. Plant Physiol. 230 (2018) 40–50, https://doi.org/10.1016/j.jplph.2018.07.013.

[11] V. Sinisi, P. Pelagatti, M. Carcelli, A. Migliori, L. Mantovani, L. Righi, G. Leonardi, S. Pietarinen, C. Hubsch, D. Rogolino, A green approach to copper-containing pesticides: antimicrobial and antifungal activity of brochantite supported on lignin for the development of biobased plant protection products, ACS Sustain. Chem. Eng. 7 (3) (2019) 3213–3221, https://doi.org/10.1021/acssuschemeng.8b05135.

[12] Z. Strassberger, S. Tanase, G. Rothenberg, The pros and cons of lignin valorisation in an integrated biorefinery, RSC Adv. 4 (48) (2014) 25310–25318, https://doi.org/10.1039/c4ra04747h.

[13] A. Khan, M. Haris, T. Hussain, A.A. Khan, S.E. Laasli, R. Lahlali, F. Mokrini, Counter-attack of biocontrol agents: environmentally benign approaches against root-knot nematodes (*Meloidogyne* spp.) on agricultural crops, Heliyon 9 (11) (2023) e21653, https://doi.org/10.1016/j.heliyon.2023.e21653.

[14] D.S. Bajwa, G. Pourhashem, A.H. Ullah, S.G. Bajwa, A concise review of current lignin production, applications, products and their environment impact, Ind. Crop. Prod. 139 (2019) 111526, https://doi.org/10.1016/j.indcrop.2019.111526.

[15] A.J. Ragauskas, G.T. Beckham, M.J. Biddy, R. Chandra, F. Chen, M.F. Davis, B. H. Davison, R.A. Dixon, P. Gilna, M. Keller, P. Langan, A.K. Naskar, J.N. Saddler, T. J. Tschaplinski, G.A. Tuskan, C.E. Wyman, Lignin valorization: improving lignin processing in the biorefinery, Science 344 (6185) (2014), https://doi.org/10.1126/science.1246843.

[16] A.C. Cassoni, I. Mota, P. Costa, M.W. Vasconcelos, M. Pintado, Effect of alkaline and deep eutectic solvents pretreatments on the recovery of lignin with antioxidant activity from grape stalks, Int. J. Biol. Macromol. 220 (2022) 406–414.

[17] A.C. Cassoni, A.I. Bourbon, L. Pastrana, M. Vasconcelos, M. Pintado, From grape stalks to lignin nanoparticles: a study on extraction scale-up, solubility enhancement and green nanoparticle production, Foods 14 (24) (2025) 4274.

[18] C. Maleita, I. Esteves, J.M.S. Cardoso, M.J. Cunha, R.M.D.G. Carneiro, I. Abrantes, *Meloidogyne luci*, a new root-knot nematode parasitizing potato in Portugal, Plant Pathol. 67 (2018) 366–376.

[19] R.S. Hussey, K.R. Barker, A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique, Plant Dis. Report. 57 (12) (1973) 1025–1028.

[20] X. Dong, M. Dong, Y. Lu, A. Turley, T. Jin, C. Wu, Antimicrobial and antioxidant activities of lignin from residue of corn stover to ethanol production, Ind. Crop. Prod. 34 (3) (2011) 1629–1634.

[21] S.E. Klein, A. Alzagameem, J. Rumpf, I. Korte, J. Kreyenschmidt, M. Schulze, Antimicrobial activity of lignin-derived polyurethane coatings prepared from unmodified and demethylated lignins, Coatings 9 (8) (2019), https://doi.org/10.3390/coatings9080494.

[22] A.G. Whitehead, J.R. Hemming, A comparison of some quantitative methods of extracting small vermiform nematodes from soil, Ann. Appl. Biol. 55 (1965) 25–38, https://doi.org/10.1111/j.1744-7348.1965.tb07864.x.

[23] W.S. Abbott, A method of computing the effectiveness of an insecticide, J. Econ. Entomol. 18 (1925) 265–267.

[24] G. Banfi, M. Del Fabbro, Biological variation in tests of hemostasis, Semin. Thromb. Hemost. 34 (7) (2008) 635–641, https://doi.org/10.1055/s-0028-1104541.

[25] J. Eisenback, Detailed morphology and anatomy of second stage juveniles, males and females of genus *Meloidodyne* (root-knot nematodes) an advanced treatise on Meloidogyne, Meloidogyne Morphology and Anatomy 1 (1985) 47–78.

[26] C. Escobar, M. Barcala, J. Cabrera, C. Fenoll, Overview of root-knot nematodes and giant cells, in: Advances in Botanical Research, vol. 73, Elsevier Ltd, 2015, https://doi.org/10.1016/bs.abr.2015.01.001.

[27] N. Aissani, R. Balti, H. Sebai, Potent nematicidal activity of phenolic derivatives on *Meloidogyne incognita*, J. Helminthol. 92 (6) (2018) 668–673, https://doi.org/10.1017/S0022149X17000918.

[28] P. Nigam, I.M. Banat, D. Singh, R. Marchant, Microbial process for the decolorization of textile effluent containing azo, diazo and reactive dyes, Process Biochemistry 31 (5) (1996) 435–442, https://doi.org/10.1016/0032-9592(95)00085-2.

[29] P. Ohri, S.K. Pannu, Effect of phenolic compounds on nematodes- A review, Journal of Applied and Natural Science 2 (2) (2010) 344–350, https://doi.org/10.31018/jans.v2i2.144.

[30] D. Kalaiselvi, A. Mohankumar, G. Shanmugam, G. Thiruppathi, S. Nivitha, P. Sundararaj, Altitude-related changes in the phytochemical profile of essential oils extracted from *Artemisia nilagirica* and their nematicidal activity against *Meloidogyne incognita*, Ind. Crop. Prod. 139 (2019) 111472, https://doi.org/10.1016/j.indcrop.2019.111472.

[31] X. Cheng, X. Liu, H. Wang, X. Ji, K. Wang, M. Wei, K. Qiao, Effect of emamectin benzoate on root-knot nematodes and tomato yield, PLoS One 10 (10) (2015) 1–9, https://doi.org/10.1371/journal.pone.0141235.

[32] J.M.S. Faria, L. Rusinque, M.L. Inácio, Nematicidal activity of volatiles against the rice root-knot nematode and environmental safety in comparison to traditional nematicides, Plants 13 (15) (2024), https://doi.org/10.3390/plants13152046.

[33] S. Laquale, P. Avato, M.P. Argentieri, M.G. Bellardi, T. D'Addabbo, Nematotoxic activity of essential oils from *Monarda* species, J. Pest. Sci. 91 (3) (2018) 1115–1125, https://doi.org/10.1007/s10340-018-0957-1.

[34] C. Maleita, I. Esteves, R. Chim, L. Fonseca, M.E.M. Braga, I. Abrantes, H.C. De Sousa, Naphthoquinones from walnut husk residues show strong nematicidal activities against the root-knot nematode *Meloidogyne hispanica*, ACS Sustain. Chem. Eng. 5 (4) (2017) 3390–3398, https://doi.org/10.1021/acssuschemeng.7b00039.

[35] N. Ntalli, N. Monokrousos, C. Rumbos, D. Kontea, D. Zioga, M.D. Argyropoulou, U. Menkissoglu-Spiroudi, N.G. Tsiropoulos, Greenhouse biofumigation with *Melia azedarach* controls *Meloidogyne* spp. and enhances soil biological activity, J. Pest. Sci. 91 (1) (2018) 29–40, https://doi.org/10.1007/s10340-017-0909-1.

[36] W.A. Cromwell, J. Yang, J.L. Starr, Y.K. Jo, Nematicidal effects of silver nanoparticles on root-knot nematode in bermudagrass, J. Nematol. 46 (3) (2014) 261–266.

[37] K. Nazir, T. Mukhtar, H. Javed, In vitro effectiveness of silver nanoparticles against root-knot nematode (*Meloidogyne incognita*), Pakistan J. Zool. 51 (6) (2019) 2077–2083.

[38] A. Khan, M.F. Bani Mfarrej, M. Danish, M. Shariq, M.F. Khan, M.S. Ansari, M. Hashem, S. Alamri, F. Ahmad, Synthesized copper oxide nanoparticles via the green route act as antagonists to pathogenic root-knot nematode, *Meloidogyne incognita*, Green Chem. Lett. Rev. 15 (3) (2022) 491–507, https://doi.org/10.1080/17518253.2022.2096416.

[39] A.A. Heflish, A.E. Hanfy, M.J. Ansari, E.S. Dessoky, A.O. Attia, M.M. Elshaer, M. K. Gaber, A. Kordy, A.S. Doma, A. Abdelkhalek, S.I. Behiry, Green biosynthesized silver nanoparticles using *Acalypha wilkesiana* extract control root-knot nematode, J. King Saud Univ. Sci. 33 (6) (2021) 101516, https://doi.org/10.1016/j.jksus.2021.101516.

[40] V. Arumugam, A.H. Bhat, I.K.S. Savarirayan, F.S. Ataya, D. Fouad, Root-knot nematode suppression through biogenic silver nanoparticles: a promising path for sustainable agriculture, J. Nanoparticle Res. 26 (11) (2024) 1–27, https://doi.org/10.1007/s11051-024-06160-7.

[41] K. Rani, N. Devi, P. Banakar, P. Kharb, P. Kaushik, Nematicidal potential of green silver nanoparticles synthesized using aqueous root extract of *Glycyrrhiza glabra*, Nanomaterials 12 (17) (2022), https://doi.org/10.3390/nano12172966.

[42] P. Figueiredo, K. Lintinen, J.T. Hirvonen, M.A. Kostiainen, H.A. Santos, Properties and chemical modifications of lignin: towards lignin-based nanomaterials for biomedical applications, Prog. Mater. Sci. 93 (2018) 233–269.

[43] W.D.H. Schneider, A.J.P. Dillon, M. Camassola, Lignin nanoparticles enter the scene: a promising versatile green tool for multiple applications, Biotechnol. Adv. 47 (2021) 107685.

[44] D. Kalaiselvi, A. Mohankumar, G. Shanmugam, S. Nivitha, P. Sundararaj, Green synthesis of silver nanoparticles using latex extract of *Euphorbia tirucalli*: a novel approach for the management of root knot nematode, *Meloidogyne incognita*, Crop Prot. 117 (2019) 108–114, https://doi.org/10.1016/j.cropro.2018.11.020.

[45] D. Orion, G. Kritzman, S.l.F. Meyer, E.F. Erbe, D.J. Chitwood, A role of the gelatinous matrix in the resistance of root-knot nematode (*Meloidogyne* spp.) eggs to microorganisms, J. Nematol. 33 (2001) 203–207.

[46] F. Daramola, N. Lewu, J. Nkiko, F. Lewu, Nematicidal effects of silver nanoparticles (AG-NPs) on the root-knot nematode, *Meloidogyne javanica* associated with Swiss chard (*Beta vulgaris* L.), Helminthologia 60 (2) (2023) 189–195, https://doi.org/10.2478/helm-2023-0018.