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# Acaricidal activity of geraniol-loaded lignin nanoparticles for the control of *Brevipalpus chilensis*: an eco-friendly approach to crop protection†

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The environmental pollution and health risks associated with synthetic pesticides have driven increasing interest in plant-derived biopesticides like geraniol. However, their practical application is limited by high volatility and low solubility. In this study, lignin nanoparticles were used as a carrier system to enhance the stability and acaricidal efficacy of geraniol against *Brevipalpus chilensis*. The nanoprecipitation process enabled the synthesis of spherical geraniol-loaded lignin nanoparticles with an average size of  $200 \pm 27.2$  nm, a surface charge of  $-29 \pm 3.9$  mV and an encapsulation efficiency of 46.5%. The release profile of encapsulated geraniol was assessed, and UV exposure assays demonstrated significantly improved stability compared to free geraniol. Bioassays revealed significantly higher mortality rates of *Brevipalpus chilensis* when treated with geraniol-loaded nanoparticles compared to free geraniol, highlighting the enhanced efficacy of the encapsulated compound. Additionally, nanoparticle formulations exhibited low cytotoxicity in HeLa cells. Overall, this study underscores the potential of lignin nanoparticles as a promising delivery system for optimizing biopesticide formulations in sustainable agriculture.

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## Environmental significance

Innovative pest control strategies that improve the efficacy of botanical biopesticides are essential for advancing an environmentally friendly agriculture. Nanocarriers have emerged as a promising technology to minimize biopesticide degradation and volatility, thereby increasing the biological activity of these natural compounds. This study underscores the potential of lignin nanoparticles as a delivery nano-system for biopesticides, utilizing the biodegradable, non-toxic and UV-shielding properties of lignin. The nanoparticles developed in this study improved the acaricidal performance of geraniol against a vineyard pest, supporting the development of efficient, safer and sustainable pest control alternatives to reduce the footprint of synthetic pesticides. Furthermore, this work contributes to the understanding of the impact of nanotechnology in agriculture.

## 1. Introduction

The widespread environmental pollution, biodiversity loss, and human health risks associated with synthetic pesticides have driven growing interest in plant-derived biopesticides for promoting sustainable agriculture.<sup>1</sup> Unlike conventional

pesticides, botanical biopesticides offer advantages such as biodegradability, host specificity, lower persistence, and reduced pesticide residue accumulation. Among the most extensively studied biopesticides are the terpenoids, phenolic compounds, and alkaloids.<sup>2,3</sup>

Geraniol (3,7-dimethylocta-*trans*-2,6-dien-1-ol), an acyclic monoterpene alcohol found in various essential oils, has demonstrated fungicidal, insecticidal, acaricidal, and insect-repellent properties.<sup>4,5</sup> Commercially available geraniol-based formulations are currently available for crop protection,<sup>6</sup> which are used for controlling mites such as *Brevipalpus chilensis* Baker (Acari: Tenuipalpidae), a pest known to negatively impact grapevine crop performance and economic yields in countries like Chile.<sup>7</sup> However, despite the potential of botanical formulations, their large-scale adoption by farmers remains limited due to challenges such as high

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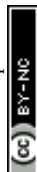
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volatility, low aqueous solubility, and degradation under environmental conditions. These factors reduce the efficacy of the botanical biopesticides, increasing the need for frequent applications and highlighting the necessity for improved formulations to enhance the performance of compounds like geraniol.<sup>8</sup>

Nanoscale materials have shown promising potential across several fields including agriculture.<sup>9,10</sup> In this regard, polymeric nanoparticles (NPs) have emerged as an innovative tool for addressing the limitations associated with the agricultural application of biopesticides.<sup>11,12</sup> Specifically, NPs have been shown to enhance the biological activity of active compounds by improving their solubility, providing sustained release, and protecting them from degradation under environmental conditions. As a result, significant efforts have been made to develop nanostructured systems that optimize biopesticide efficacy.<sup>13–15</sup> Among the various biopolymers employed for NP synthesis, lignin stands out due to its biocompatibility, biodegradability, natural ultraviolet (UV)-shielding properties, and high thermal stability.<sup>16,17</sup> Moreover, lignin NPs (LNPs) effectively encapsulate hydrophobic molecules, making them attractive carriers for poorly soluble compounds. Additionally, using LNPs is considered an environmentally friendly approach, adding value to lignin, the second most abundant biopolymer on earth.<sup>18</sup> Here, this study aimed to develop and characterize an eco-friendly nanosystem based on LNPs loaded with geraniol (LNPs-G) and to assess their acaricidal effects against *Brevipalpus chilensis*, contributing to sustainable and secure agricultural practices.

## 2. Experimental

### 2.1 Materials

Lignin, alkali (5% moisture), geraniol (98% purity), Tween 80, acetone (for analysis), acetonitrile (ACN, HPLC grade), and methanol (for analysis) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

### 2.2 Nanoprecipitation process

LNPs-G were synthesized using a nanoprecipitation method followed by solvent evaporation, as described previously with certain modifications.<sup>19</sup> Briefly, a 2 mg mL<sup>-1</sup> of alkali lignin solution was prepared in a mixture of acetone: deionized water (9:1). The mixture was filtered using a 0.45 µm syringe filter and adjusted to pH 8. Then, geraniol was added in a concentration of 2.5% v/v. A volume of 4.5 mL of the lignin/geraniol solution was added dropwise against ultrapure water using a syringe pump (NE-1000, New Era Pump Systems, USA) at a rate of 1.5 mL min<sup>-1</sup>, with constant stirring until complete acetone evaporation. The resulting LNPs-G were stored at -80 °C for 48 h and lyophilized for three days (BK-FD-10S, BIOBASE, China). Unloaded LNPs (LNPs-empty) were prepared similarly without the addition of geraniol, to be used as controls. Lyophilized LNPs were resuspended in Milli-Q and maintained for 15 minutes in an ultrasonic bath (Elmasonic S10H, Elma, Germany) for their physicochemical characterization.<sup>20</sup>

### 2.3 Physicochemical characterization of LNPs-G

**2.3.1 Hydrodynamic diameter, polydispersity index (PDI), and zeta (ζ) potential.** The size (nm) and PDI of the synthesized LNPs were determined using dynamic light scattering (DLS) with a Malvern Zetasizer Nano-S90 (Malvern Instruments, UK). The ζ potential (surface charge) was determined by electrophoretic light scattering (ELS) using a Zetasizer Nano ZSP 3000 (Malvern Instruments, UK).<sup>21</sup> The measurements were obtained in duplicate at 25 °C using 1 mL of the LNP suspension, and the results from eight determinations were reported as mean diameter ± standard deviation (mean ± SD; nm).

**2.3.2 Morphology of LNPs.** The morphology of LNPs-G and LNPs-empty from three independent preparations was analyzed using scanning transmission electron microscopy (STEM) with an FE-SEM INSPECT-F50 (FEI, USA). 10 µL of the LNP suspension (0.01 mg mL<sup>-1</sup>) was deposited onto a copper grid, followed by the addition of 10 µL of ultrapure water and staining with uranyl acetate at 22 °C. The samples were then dried for 24 h, subjected to gold sputtering, and imaged at an accelerating voltage of 10 kV with a working distance of 5 mm.

**2.3.3 Quantitative analysis of geraniol.** Geraniol was quantified using ultraperformance liquid chromatography (UPLC, Acquity Systems–Waters Corp., USA) with a Waters Acquity C18 column (50 × 2.1 mm, 1.7 µm). The mobile phase consisted of acetonitrile (ACN) and water (60:40, v/v), operating at a flow rate of 1 mL min<sup>-1</sup> at 25 °C. A 10 µL injection volume was employed, and the detection was performed at a wavelength of 210 nm. The calibration curve was constructed with geraniol concentrations ranging from 0.0005 to 0.5 mg mL<sup>-1</sup>; a linearity coefficient of 0.999, and a quantification limit of 100 ng mL<sup>-1</sup> (Fig. S1†).<sup>22</sup>

**2.3.4 Encapsulation efficiency and the cumulative release of geraniol.** The encapsulation efficiency (EE%) of geraniol in LNPs was determined using an extraction method.<sup>23</sup> Briefly, 10 mg of lyophilized LNPs-G were dissolved in ACN, followed by intense agitation for 1 h with rotary incubation at 22 °C for 24 h (Roto-Therm Mini, Benchmark Scientific Inc., USA). The geraniol concentration was determined by UPLC, and the EE% was calculated using the following equation:

$$EE\% = \frac{\text{Weight of geraniol after extraction}}{\text{Initial weight of geraniol}} \times 100$$

The cumulative release of geraniol from LNPs-G was assessed using the rapid equilibrium dialysis system (RED, Thermo Scientific, USA). LNPs-G were resuspended in an aqueous solution containing 5% (v/v) Tween 80 to enhance geraniol solubility in water. 500 µL of the LNPs-G suspension (0.5 mg mL<sup>-1</sup> of geraniol) was loaded into the sample compartment of the dialysis membrane system, while 750 µL of the same aqueous/Tween 80 solution was added to the opposing compartment. The dialysis system was sealed and maintained under constant agitation in an orbital shaker at 25 °C. Samples from the opposing compartment were collected at predeterminate time intervals (3 h, 6 h, 12 h, 18 h, 24 h, 72 h, 96 h, 120 h, 144 h, 168 h and 192), replaced with fresh solutions



and quantified by UPLC. Four independent LNPs-G suspensions at the same concentration were tested.<sup>24</sup>

**2.3.5 Fourier-transform infrared spectroscopy (FT-IR) analysis.** The chemical structure of freeze-dried LNPs-G was characterized using FT-IR on a IFS66V spectrometer (BRUKER, Germany). The FT-IR spectra were acquired in the mid-infrared range (500–4000  $\text{cm}^{-1}$ ), with a spectral resolution of 4  $\text{cm}^{-1}$ . A total of 32 scans were accumulated per sample to enhance the signal/noise ratio and improve data quality. The FT-IR spectra of all samples were recorded under identical conditions, and all measurements were performed in triplicate. The FT-IR spectra of LNPs-G were compared with those of free geraniol and LNPs-empty.

#### 2.4 Photostability assays

LNPs' encapsulation capability to protect geraniol from photodegradation was evaluated by exposing 1.5 mL of three independent suspensions of LNPs-G (geraniol concentration of 0.5  $\text{mg mL}^{-1}$ ) to UV light (365 nm) in a dark chamber at 25 °C under constant agitation in an orbital shaker. The samples were collected at 3 h, 6 h, 9 h, 12 h, 18 h, and 24 h; geraniol extraction and quantification were performed using the method described in section 2.3.3, and the results were compared to free geraniol. Free geraniol, prepared in 5% (v/v) of Tween 80° at a concentration equivalent to that encapsulated in LNPs-G was exposed to the same conditions. LNPs and free geraniol were stored under dark conditions as controls for the assay.

#### 2.5 Bioassays to evaluate acaricidal effect of LNPs-G on *Brevipalpus chilensis*.

The acaricidal effects of LNPs-G, free geraniol and LNPs-empty were evaluated over *Brevipalpus chilensis*. The specimens were obtained from *Ligustrum lucidum* plants and transferred to experimental units consisting of two 2 cm leaf disks placed into a moistened cotton in a 10 cm Petri dish. Each leaf disk was infested with 10 female adult mites and sprayed with geraniol encapsulated in concentrations ranging from 0.0005  $\text{mg mL}^{-1}$  to 5  $\text{mg mL}^{-1}$ , and for this purpose, LNPs-G were suspended considering the EE% of geraniol. Mortality was evaluated at 24 h, 48 h, 72 h, and 96 h post-treatment under a microscope within 20× magnification, and the results were compared to those of free geraniol and LNPs-empty (in concentrations equivalent to those of encapsulated geraniol and LNPs-G, respectively). The mites were considered dead when movement was not observed upon touching with a brush hair. The mortality rates were calculated and corrected using Abbott's formula.<sup>25</sup> The experimental units were retained at 26 °C  $\pm$  2 °C under conditions of 65  $\pm$  15% relative humidity and a 16 h/8 h light/dark cycle. The data were expressed as the calculated area under the curve (AUC) from the mortality percentage of preparations over time, and it was expressed as the [Log]  $\text{mg mL}^{-1}$  of geraniol to calculate the  $\text{IC}_{50}$  value.

#### 2.6 Viability of HeLa cells

The viability of HeLa cells was assessed using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay (Biotium, USA), following the manufacturer's instructions. Briefly, 7000 cells were seeded per well into a 96-well plate and incubated at 37 °C in a humidified atmosphere containing 5%  $\text{CO}_2$  until 90% confluence. The cells were then treated with various concentrations of LNPs-G (0.0002–20  $\text{mg mL}^{-1}$  of geraniol), free geraniol (at an equivalent concentration of encapsulated geraniol), or LNPs-empty and incubated for 24 h. Subsequently, 10  $\mu\text{L}$  of the MTT solution was added to each well and incubated for 4 h. DMSO was then added, and absorbance was measured at 570 and 630 nm using a multiplate reader (EPOCH, BIOTEK, USA). Untreated cells and wells containing the Dulbecco's modified Eagle medium (DMEM, Corning, USA) but lacking cells were employed as controls corresponding to 100% and 0% viability, respectively. Six independent assays were performed, and the data were analyzed using two-way ANOVA and *t*-tests (GraphPad Software Inc., San Diego, CA).<sup>26</sup>

#### 2.7 Data analysis

The data are presented as mean  $\pm$  standard deviation (SD). Statistical analysis was performed using GraphPad Prism software (version 8.0, GraphPad Software Inc., San Diego, CA). Differences between the groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test for multiple comparisons. A *p*-value less than 0.05 was considered statistically significant.

### 3. Results and discussion

#### 3.1 Preparation and characterization of LNPs-G

LNP formulations were synthesized by nanoprecipitation without the use of stabilizing agents; compared to other conventional techniques, this methodology is low cost and reproducible, which is crucial for scalability, an ongoing challenge in agricultural nanotechnology.<sup>27</sup> After the standardization of the synthesis parameters (Fig. S2 and S3 and Table. S1†), the LNP suspensions were characterized post-synthesis by DLS, ELS, and STEM. Table 1 summarizes the results obtained for the hydrodynamic diameter, PDI, and surface charge determined through cumulant analysis. The LNPs-empty showed an average particle size of 134  $\pm$  17.0 nm, and the incorporation of geraniol resulted in a significant increase in the particle size to 200  $\pm$  27.2 nm, suggesting the incorporation of geraniol into the LNP core with an EE% of 46.5%. In addition, a significant difference in the PDI was not observed between LNPs loaded with geraniol and LNPs-empty. The LNPs are characterized to exhibit a high negative surface charge, attributable to the presence of phenolic and hydroxyl (–OH) groups on lignin monomers.<sup>28,29</sup> As we anticipated, the surface charge of the formulations was  $-29.4 \pm 3.9$  mV for LNPs-G and  $-32.4 \pm 3.4$  mV for LNPs-empty, which suggests an optimal electrostatic



**Table 1** Determination of the diameter, PDI, and  $\zeta$  potential of the synthesized LNPs

|                        | Diameter $\pm$ SD (nm)      | PDI $\pm$ SD                 | $\zeta$ potential $\pm$ SD (mV) | EE%  |
|------------------------|-----------------------------|------------------------------|---------------------------------|------|
| LNPs-empty ( $n = 8$ ) | 134 $\pm$ 17.0 <sup>a</sup> | 0.31 $\pm$ 0.03 <sup>a</sup> | -32 $\pm$ 3.4 <sup>a</sup>      | —    |
| LNPs-G ( $n = 8$ )     | 200 $\pm$ 27.2 <sup>a</sup> | 0.33 $\pm$ 0.06 <sup>a</sup> | -29 $\pm$ 3.9 <sup>a</sup>      | 46.5 |

<sup>a</sup> Indicates statistically significant differences within the same column ( $p < 0.05$ ).

repulsion between the particles, which indicates a stable aqueous colloidal suspension formulated by the nanoprecipitation process.<sup>30,31</sup> Both (geraniol and empty) LNP formulations exhibited a monomodal size distribution and spherical morphology, observed by STEM. Additionally, the LNPs-G demonstrated a bigger particle size compared to LNPs-empty, consistent with the results of DLS analysis (Table 1 and Fig. 1).

The normalized FT-IR spectra of LNPs-G, LNPs-empty, and free geraniol were obtained for comparing their chemical structures (Fig. 2). The FT-IR spectrum of free geraniol exhibited characteristic O–H stretching vibrations at 3326  $\text{cm}^{-1}$ , C–H stretching vibrations corresponding to alkanes at 2974, 2923, and 2850  $\text{cm}^{-1}$ , and C=C stretching vibrations at 1675 and 1442  $\text{cm}^{-1}$ . The deformation of the C–H vibrations from the methyl ( $\text{CH}_3$ ) groups were observed at 1383  $\text{cm}^{-1}$ , along with the C–O stretching vibrations at 996  $\text{cm}^{-1}$ . The spectrum of LNPs-empty showed the expected profile, with O–H vibrations at 375  $\text{cm}^{-1}$ , the C–H stretching corresponding to the methyl groups at 2983  $\text{cm}^{-1}$ , and the aromatic skeletal vibrations at 1598  $\text{cm}^{-1}$ .<sup>32,33</sup> Additional bands at 1030 and 830  $\text{cm}^{-1}$  correspond to the in-plane and out-of-plane C–H deformations of the aromatic ring, respectively.

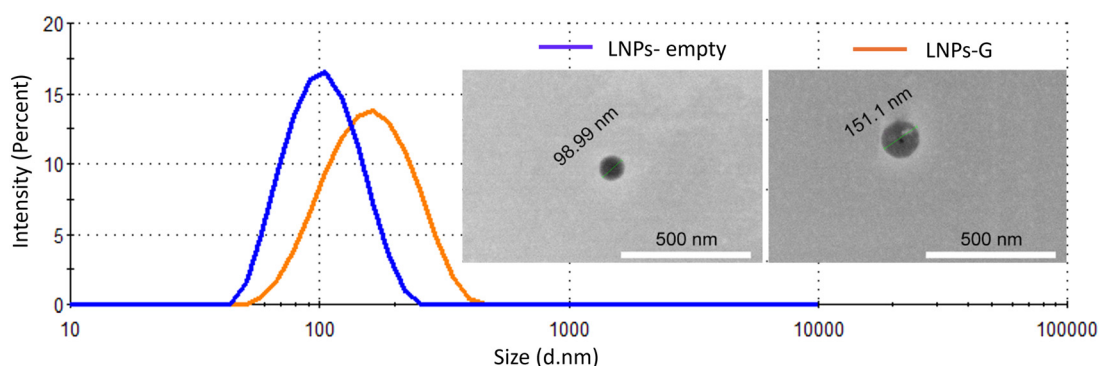
In contrast, the FT-IR spectrum of LNPs-G did not exhibit any vibrations associated with free geraniol, and no differences were observed when compared with the spectrum of LNPs-empty, suggesting that geraniol is not present on the surface of the NPs. This result is consistent with the freeze-drying process used for NP preparation, which may have resulted in the removal of any unbound geraniol molecules.

### 3.2 Cumulative release profile of geraniol from LNPs-G

To explore the cumulative release profile of geraniol from LNPs-G, the rapid equilibrium dialysis system was used. Notably, 17  $\pm$  5% of the total geraniol incorporated into the LNPs was released within the first 3 h of dialysis, likely due to the diffusion of molecules near the surface of the LNPs-G (Fig. 3). Additionally, 50  $\pm$  2% and 95  $\pm$  3% of the geraniol was released after 16 and 72 h of dialysis, respectively. de Oliveira *et al.*<sup>15</sup> and Yegin *et al.*<sup>34</sup> reported the release of 50% of geraniol from chitosan/arabic gum-based NPs within 1.7 h and from Pluronic® F127 NPs after 7.25 h of dialysis, respectively; these data suggest that the release of geraniol varies depending on the polymer used for the synthesis of nanoparticles.<sup>35</sup> Studies on essential oils encapsulated within LNPs have revealed a sustained release of essential oils from *Thymus* spp. over a seven-day duration and maximum release of 44.2% (without achieving full release) for the essential oils from *Rosa damascena*.<sup>36</sup> These observations underscore the potential applications of LNPs for pest control, given that sustained release is a critical feature for reducing the required doses of active compounds.

### 3.3 Photostability assays

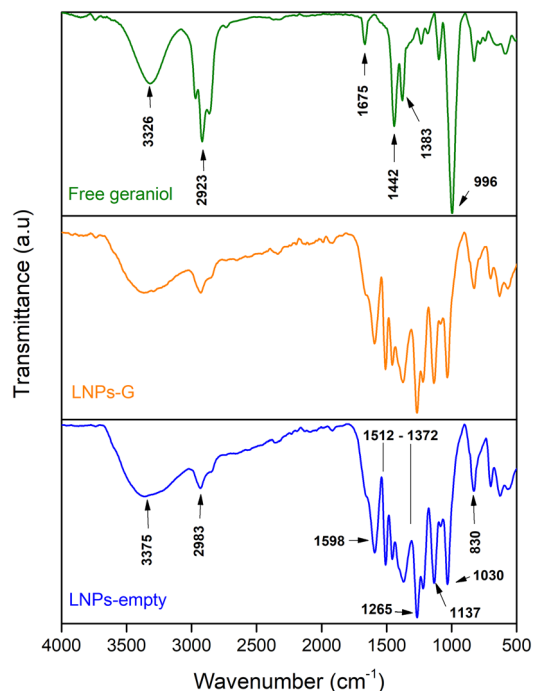
Botanical compounds are known for their rapid biodegradation, short persistence, and low accumulation of residues associated with UV exposure, which can significantly limit their biological efficacy under field conditions.<sup>2,37</sup> Considering the above limitations, a UV exposure assay was conducted to determine whether the incorporation of geraniol into LNPs enhances its photostability.



**Fig. 1** Size distribution and morphology of LNPs. Representative size distribution profiles obtained by dynamic light scattering (DLS) for LNPs-empty (blue line) and LNPs-G (red line). Spherical morphologies of both LNP formulations, visualized by STEM, are presented in the accompanying panels. A scale bar of 500 nm and a magnification of 100 000 $\times$  were used for imaging. In both cases, the size of LNPs-G was higher than that of LNPs-empty.

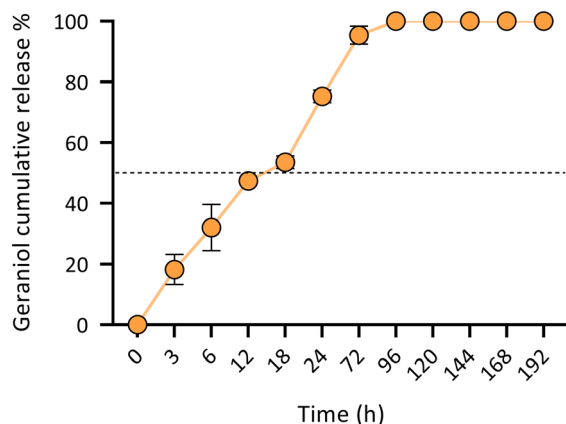




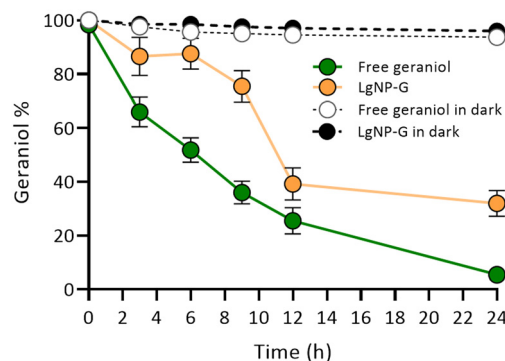


**Fig. 2** Normalized FT-IR spectra of free geraniol, geraniol-loaded LNP-G, and unloaded LNP-G (LNP-G-empty), measured over the range 500–4000  $\text{cm}^{-1}$ . LNP-G and LNP-G-empty showed a similar spectrum. A total of 32 accumulated scans were performed per sample. All samples were analyzed in triplicate.

Free geraniol exhibited 50% of degradation after 6.1 h of UV exposure, whereas the LNP-G-encapsulated geraniol displayed the same level of degradation only after 11.2 h (Fig. 4). After 24 h of UV exposure, free geraniol was completely degraded, whereas the LNP-G-encapsulated geraniol retained 40% of its initial content, demonstrating its protective effect. Additionally, neither free geraniol nor LNP-G exhibited degradation over time when



**Fig. 3** Cumulative release of geraniol from LNP-G upon dialysis at 25  $^{\circ}\text{C}$ . Geraniol release of 50% (dotted line) and a total (100%) release were attained after 16 h and 96 h of dialysis, respectively. Data represent mean  $\pm$  SD of four independent assays.

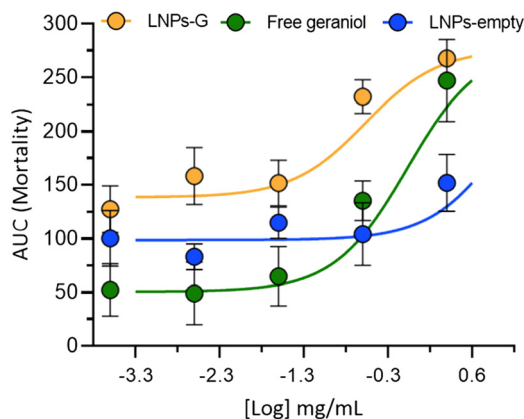


**Fig. 4** LNP-G and free geraniol were exposed to UV irradiation (365 nm) for 24 hours at 25  $^{\circ}\text{C}$ . Geraniol encapsulated within LNP-G exhibited significantly lower degradation compared to free geraniol, with 50% degradation occurring at 11.2 hours and 6.2 hours, respectively. Additionally, the results obtained under dark conditions are represented by dotted lines. Data are expressed as mean  $\pm$  SD from three independent assays.

stored in darkness during the assay. The enhanced stability of geraniol upon encapsulation within LNP-G can be attributed to the UV-shielding properties of lignin, associated with its multiple chromophore and achromatic groups, including conjugated phenols, carbonyl groups, and conjugated double bonds, that allow absorption of UV light at 250–400 nm.<sup>38</sup>

### 3.4 Bioassays to evaluate the acaricidal activity of LNP-G on *Brevipalpus chilensis*

The acaricidal activity of LNP-G, free geraniol and LNP-G-empty against *Brevipalpus chilensis* was evaluated based on the  $\text{IC}_{50}$  value of geraniol (Fig. 5). The results indicated that LNP-G exhibited an  $\text{IC}_{50}$  of 0.53  $\text{mg mL}^{-1}$ , whereas free geraniol and LNP-G-empty showed  $\text{IC}_{50}$  values of 1.70  $\text{mg mL}^{-1}$  and 24.25  $\text{mg mL}^{-1}$ , respectively. These findings suggest that encapsulating geraniol within LNP-G enhances its acaricidal effect. Moreover, LNP-G demonstrated significantly higher acaricidal activity 48 h post-application, implying that the efficacy of geraniol is potentiated overtime (Fig. S4†). Similarly, Campos *et al.*<sup>39</sup> reported the acaricidal effect of nanoencapsulated botanicals against *Tetranychus urticae* at 12, 24, 48, and 72 h post-application. Additionally, Ebadollahi *et al.*<sup>40</sup> found that essential oils from *Thymus eriocalyx* were more effective when encapsulated in a nanometric mesoporous material than their non-encapsulated counterparts. The increased efficacy of encapsulated bioactive compounds is generally attributed to their higher bioavailability.<sup>41,42</sup> In the case of geraniol, this enhanced bioavailability may result from increased stability, reduced degradation, and sustained release upon encapsulation within LNP-G. Notably, LNP-G-empty exhibited an acaricidal effect like LNP-G at low concentrations, suggesting that LNP-G themselves contribute to *Brevipalpus chilensis*' mortality. This observation aligns with studies that highlights the intrinsic bioactive properties of lignin-based biopolymers.<sup>43–45</sup>



|               | Log IC <sub>50</sub> | IC <sub>50</sub> (mg/mL) |
|---------------|----------------------|--------------------------|
| LNP-G         | -0.26                | 0.53                     |
| Free geraniol | 0.23                 | 1.70                     |
| LNP-empty     | 1.38                 | 24.25                    |

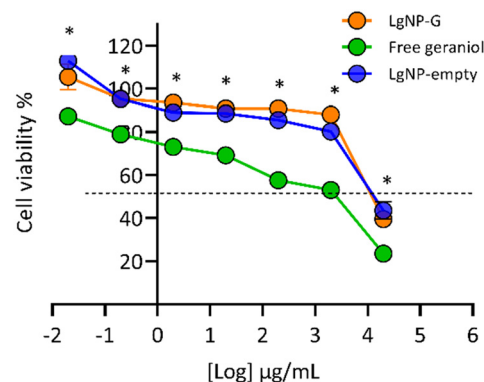
**Fig. 5** IC<sub>50</sub> determination for LNP-G, free geraniol, and LNP-empty. The area under the curve (AUC) was calculated based on the percentage mortality associated with LNP-G, free geraniol and LNP-empty following their evaluation against *Brevipalpus chilensis* over 96 h. The results suggest that LNP-G exhibit greater efficacy in delivering geraniol encapsulated in LNP-G compared to the free compound. Data are expressed as mean  $\pm$  SD of the AUC of mortality and seven independent assays.

### 3.5 Cell viability assays

A cytotoxicity assay was conducted to assess whether the synthesized LNP-G affect the viability of HeLa cells. Free geraniol and LNP-empty were used as controls for comparison. Cell viability remained above 80% following the treatment with LNP-G and LNP-empty at concentrations up to 2000  $\mu\text{g mL}^{-1}$  (equivalent to 3.3 on the logarithmic scale). In contrast, at this same concentration, free geraniol reduced cell viability to 50%, indicating significantly higher cell toxicity across all the tested concentrations (Fig. 6). These findings suggest that encapsulating geraniol within LNPs mitigates its potential toxicity. Similar results were reported by Zhou *et al.*, 2019,<sup>46</sup> who reported that LNPs had no adverse effects on HeLa cell viability. Furthermore, these results are consistent with the well-documented biocompatibility of LNPs, which is a crucial factor for developing safe and effective formulations for agricultural pest control with minimal toxicity.<sup>47,48</sup>

## 4. Conclusion

Lignin nanoparticles have been widely recognized for their versatility and efficiency as delivery systems for biologically active molecules. In this study, geraniol was successfully encapsulated within lignin nanoparticles using a simple and potentially scalable nanoprecipitation technique. The resulting synthesized nanoparticles improved geraniol stability under UV exposure and facilitated its sustained release, leading to enhanced acaricidal activity against *Brevipalpus chilensis* compared to free geraniol. Additionally,



**Fig. 6** Cytotoxicity of LNP-G in HeLa cells. Viability of HeLa cells following treatments with free geraniol, LNP-empty and LNP-G assessed by MTT assays ( $n = 6$ ). Statistically significant differences were not observed between LNP-empty and LNP-G treatments ( $p > 0.05$ ). However, treatment with free geraniol resulted in a significant decrease in cell viability. Data were evaluated using two-way ANOVA followed by Tukey's test.

lignin nanoparticles showed an intrinsic acaricidal effect at low concentrations compared to free geraniol and demonstrated low toxicity toward HeLa cells, supporting the reported biocompatibility of these types of nanoparticles.

In summary, this work highlights the potential of lignin nanoparticles as an effective nanocarrier for biopesticides such as geraniol, contributing to the reduction of synthetic agrochemical usage and the promotion of sustainable agriculture. However, despite the potential of lignin nanoparticles, future challenges remain, particularly regarding scalability for large-scale production and in-field evaluations.

## Data availability

The data supporting this article have been included as part of the ESI.†

## Conflicts of interest

The authors of this work declare no conflicts of interest.

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