



Interaction mechanism of plant-based nanoarchitected materials with digestive enzymes of termites as target for pest control: Evidence from molecular docking simulation and *in vitro* studies

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ABSTRACT

The integration of nanotechnology for efficient pest management is gaining momentum to overcome the challenges and drawbacks of traditional approaches. However, studies pertaining to termite pest control using bio-synthesized nanoparticles are seldom. The present study aims to highlight the following key points: a) green synthesis of AgNPs using *Glochidion eriocarpum* and their activity against wood-feeding termites, b) testing the hypothesis that AgNPs diminish digestive enzymes in termite gut through *in silico* analysis. The green synthesis route generated spherical PsAgNPs in the size range of 4–44.5 nm exhibiting higher thermal stability with minimal weight loss at 700 °C. The choice and no-choice bioassays confirmed strong repellent (80.97%) and antifeedant activity of PsAgNPs. Moreover, PsAgNPs exposure caused visible morphological changes in termites. Molecular docking simulation indicated possible attenuation of endoglucanase and bacteria-origin xylanase, digestive enzymes from termite gut, through partial blocking of the catalytic site by AgNPs. Altogether, our preliminary study suggests promising potentials of PsAgNPs for pest management in forestry and agriculture sectors to prevent damages to living trees, wood, crops, etc. As sustainable pest management practices demand low risk to the environment and biodiversity therefore, we recommend that more extensive studies should be performed to elucidate the environmental compatibility of PsAgNPs.

1. Introduction

Considering the developing biosafety concerns associated with nanotechnological applications worldwide, the green synthesis methods for generating nanoparticles are becoming popular due to their environment-friendly and sustainable approach and are much needed in the present time (Iravani, 2011; Singh et al., 2018). The green synthesis approach provides an excellent opportunity for utilizing natural biomolecules for the synthesis of nanoparticles (Mishra et al., 2014; Behravan et al., 2019). In general, naturally occurring microorganisms and plants have been identified as the best available bioagents for the same purpose (Narayanan and Sakthivel, 2010; Li et al., 2011; Gopinath et al.,

2016; Jain and Mehata, 2017; Yu et al., 2019). Nevertheless, natural biomolecules (originating from beneficial bioagents) based synthesis of nanoparticles are likely to expand their application range in various important areas for societal benefits. Therefore, plants (due to their dominance and easy accessibility) are considered as the most suitable natural resource providing a rapid, inexpensive, non-toxic and eco-friendly method that is accepted worldwide (Philip et al., 2011). Phytochemicals such as polyphenols, terpenes, tannins, flavonoids, ketones, etc. play a key role as reducing agents aiding the synthesis of nanoparticles and further provide stability via capping of the synthesized nanoparticles (Park et al., 2011; Marslin et al., 2018). Most importantly, large-scale synthesis under non-aseptic conditions, simple experimental

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setup, and high kinetics are major advantages associated with plant-mediated synthesis method.

Several plants have been explored for their ability to synthesize silver nanoparticles (AgNPs) showing antimicrobial and antiparasitic activities, for example, *Catharanthus roseus* (Mukunthan et al., 2011), *Tribulus terrestris* (Gopinath et al., 2012), *Aloe vera* (Tippayawat et al., 2016), *Clitoria ternatea* and *Solanum nigrum* (Krithiga et al., 2015), *Azadirachta indica* (Ahmed et al., 2016), *Berberis vulgaris* (Behravan et al., 2019), *Coccinia grandis* (Arunachalam et al., 2012), *Moringa oleifera* (Moodley et al., 2018), etc. Here, we report the synthesis of AgNPs using leaf extract of *Glochidion eriocarpum* Champ. ex Benth., a traditional medicinal plant. *G. eriocarpum* (Phyllanthaceae) is a shrub distributed in Asian countries such as Southern China, Thailand, Myanmar, Vietnam, Laos, etc. (Yao et al., 2018; Zhang et al., 2020). To the best of our knowledge, *G. eriocarpum* has not been explored before for the synthesis of AgNPs.

In particular, AgNPs among metallic nanoparticles have gained enormous popularity due to their antimicrobial properties indicating wide-ranging applications in many sectors (Rai et al., 2009; Burduşel et al., 2018). The promising results and anticipated benefits of AgNPs in agriculture have opened up a new avenue of research in other areas such as forestry and agroforestry, particularly for pest management purposes. Termites are known for their significant contributions to ecosystem functions such as organic matter decomposition, nutrients cycling and soil fertility in tropical and sub-tropical forests (Bignell, 2006; Jouquet et al., 2011; Govorushko, 2019; Liu et al., 2019). However, harmful species of termites are considered as pests of agricultural crops, forestry trees, and woods due to their malicious activities causing considerable economic loss globally (Junqueira and Florencio, 2018; Govorushko, 2019). Despite knowing the potential benefits of nanotechnology for pest management (Goswami et al., 2010; Worrall et al., 2018), their worth for termite pests control and promising implications in forest system is largely unexplored. To address this gap, the present study aims to (a) highlight the important role of AgNPs synthesized using green synthesis route for termite pests control, (b) improve our understanding of AgNPs beyond well-studied antimicrobial properties, and (c) explore the possible mechanisms of interaction between termite and AgNPs for improving pest management efficiency.

Higher termites possess a unique and complex digestive system where digestive enzymes secreted by termites host and gut symbionts play a key role in the metabolism of lignocellulosic materials (Brune, 2014). Since termite's survival is highly dependent on its digestive system and gut-associated microbes, therefore, targeting this system could be an important strategy for efficient pest control (Lima et al., 2014). We hypothesize that AgNPs have hazardous impacts on the digestive enzymes of termites and their gut symbionts. Considering the dearth of knowledge regarding this, we attempt to study termites-AgNPs interaction for proposing their pest management efficiency. Here, we provide evidence from laboratory-based experiments showing activities of AgNPs against termites and molecular docking analysis disclosing details of interactions between AgNPs and digestive enzymes.

2. Materials and methods

2.1. Collection of plant materials and extraction methods

Fresh, healthy, green, and medium-sized leaves of *G. eriocarpum* were collected from the plants growing in the Xishuangbanna Tropical Botanical Garden (21°41' North latitude, and 101°25' East longitude) under natural conditions in a clean plastic bag. After transporting to the laboratory, leaves were properly washed with distilled water to remove dust and other impurities. After washing, leaves were kept under the blotting paper to absorb excess water on the leaf surface. For the preparation of leaves extract, 10 g of washed fresh leaves were chopped into small pieces in a sterile glass beaker with the help of a clean scissor. To this, 100 mL of Milli-Q (MQ) water was added and boiled for 3 minutes

using a microwave oven. The extract was cooled down and filtered through Whatman filter paper no.1. The filtered leaf extract was immediately used for AgNPs synthesis.

2.2. Biosynthesis of AgNPs using *G. eriocarpum* leaves extract

For the biosynthesis of AgNPs, we followed the protocol described by Krishnaraj et al. (2010) with some modifications. The fresh stock of silver nitrate (AgNO_3) (GB670-86, Shanghai Chemical Reagent Co. Ltd.) was prepared in sterile distilled water at the final concentration of 1 mM. To 100 mL of 1 mM AgNO_3 stock in a sterile conical flask, 10 mL of leaves extract (as prepared in the previous step) was added slowly with continuous stirring of a conical flask. After this, the conical flask was covered with aluminum foil and kept in a rotary shaker (140 rpm) at 30 °C under dark condition. The biosynthesis of AgNPs was monitored by a color change of the solution to dark brown. The biosynthesized AgNPs (hereafter referred to as PsAgNPs) were harvested by centrifugation at 7000 rpm for 30 min (Dakshayani et al., 2019) followed by washing of pellet with MQ water to remove impurities.

2.3. Characterization of PsAgNPs

The UV-vis absorption spectrum of the 1 mL PsAgNPs aliquot was recorded at a wavelength range of 200–600 nm to confirm the efficient synthesis of AgNPs using UV-visible spectrophotometer (UV-2600, Shimadzu Corporation, Japan) at a resolution of 1 nm. The leaf extract diluted in the same range (i.e. 10 mL leaves extract + 100 mL water) was used to set a blank. The morphology and elemental composition of the PsAgNPs were analyzed using scanning electron microscopy (SEM) (FEI Nova NanoSEM 450, USA) equipped with the energy dispersive X-ray spectrometer (X-Max^N Oxford Instruments) at 15 kv acceleration voltage. Furthermore, morphology and size of the PsAgNPs were confirmed by transmission electron microscopy (TEM) (FEI Tecnai G2 TF30 S-Twin, USA). The X-ray diffraction (XRD) analysis for powdered PsAgNPs was done using X-ray diffractometer (Empyrean, PANalytical, The Netherlands) with Cu-K α radiation (40 kv, 40 mA) in the range of $2\theta = 20^\circ - 80^\circ$ with a wavelength of 1.54 Å. The functional characterization of PsAgNPs was done using Fourier transform infrared (FTIR) spectrometer (Bruker Tensor 27, Germany) using KBr pellets. The FTIR spectrum was recorded from 400–4000 cm^{-1} wavenumber at a resolution of 4 cm^{-1} . In addition, the Raman spectrum was also recorded to identify biomolecules attached to PsAgNPs surface using Raman spectrometer (LabRAM HR Evolution, HORIBA, Japan) in a spectral range of 100–4000 cm^{-1} . Thermogravimetric analysis (TGA) was performed to check the thermal stability of PsAgNPs by a synchronous thermal analyzer TGA/DSC HT 1600 (METTLER TOLEDO International Traditional Limited Company, Switzerland). The powdered sample of PsAgNPs was exposed to a temperature range of 25–700 °C with a heating rate of 10 °C/min under nitrogen gas atmosphere (20 mL/min).

2.4. Effect of PsAgNPs treatment on seed germination

To study whether PsAgNPs possess any toxic effect on plant germination, the seed germination assay was conducted under *in vitro* condition. The *Cajanus cajan* seeds were washed properly with water and surface dried using blotting paper. Three treatments were set as follows: control (water), AgNO_3 (50 $\mu\text{g/mL}$) and AgNPs (50 $\mu\text{g/mL}$). In total 6 replicates were maintained for each treatment. The seeds were soaked in the respective suspension for 1 h. After this, seeds were placed in a petriplate containing moist blotting paper and left at room temperature till germination starts. Seed germination was recorded in each treatment set. Primary root length and lateral root length were also measured at different time intervals to observe the pattern of growth. Another set of the same experiment (as mentioned above) was also monitored by the Plants Vivo Imaging System (Berthold Technolog, Germany) to observe the progress of growth in different treatments.

2.5. Anatomical analysis for studying silver accumulation in plant cells

The transverse sections (TS) of the primary root of each treatment (Control, AgNO₃, PsAgNPs) were cut with the help of a sharp razor and further stained with 50% safranin (Tripathi et al., 2017). After staining, TS was mounted on glycerin over the glass slide and observed under a microscope equipped with a digital camera (Leica microsystem DM2000, Germany).

2.6. Termite repellent assay (Choice bioassay)

We collected termites from the infested dead wood lying on the ground in Xishuangbanna Tropical Botanical Garden. For capturing termites, deadwood logs containing termites were collected and transported to the laboratory. After this, termites were captured gently from the deadwood using a featherweight forcep. For identification, termites were examined under the microscope and later identified as *Odontotermes* sp. The repellent activity was determined using a filter paper technique (Rech-Cainelli et al., 2015). Filter paper disc was divided into two equal parts. One-half part was soaked with water and the other half part was soaked in 1 mL of 0.1% PsAgNPs suspension. Both parts of soaked blotting paper were put in a petridish (8.5 cm diameter) maintaining a proper space between them. Then the population of 50-65 collected termites including soldiers and workers was released at the center point of the petridish. This experimental set up was replicated three times. The movement of termites was monitored at a timely interval to know which part termites like to go. Finally, the number of termites was counted on each part after 72 h and percent repellency was calculated to check the efficacy of PsAgNPs. For further validation, the bioassay was technically repeated four times and 3 replicates were maintained each time.

2.7. Contact toxicity assay (No-choice assay)

Contact toxicity assay was performed to study the direct interaction between termites and PsAgNPs in triplicates. In this assay, termites were provided no-choice and were released in the experimental chamber (same as described above) containing either PsAgNPs soaked or water-soaked filter paper disc. Termites were observed under a microscope equipped with a digital camera (Leica microsystem DFC450 C, Germany) after 24 h of treatments for viewing the morphological and structural changes. To determine the antifeedant activity, the experiment was further extended for one week and the percent antifeedant index (AI) was calculated using the formula described by Rech-Cainelli et al. (2015).

Another experimental setup was prepared to link the repellent potency of PsAgNPs with tunneling activity and antifeedant behavior of termites. An experimental chamber consists of a 100 mL glass beaker (7 cm height x 5.5 cm diameter) filled with 100 g of 25% moistened soil. Control and PsAgNPs treated chambers were maintained separately by placing a filter paper disc (5.0 cm diameter) soaked in water and PsAgNPs, respectively on top of the soil and later covered with aluminum foil after introducing 30 termites inside each chamber. We used this setup to observe the tunneling behavior of termites resulting from the repellent activity of PsAgNPs. Termite movement within the chamber was observed at a timely interval. The experiment continued for 14 days for observing tunneling response and paper consumption.

2.8. Molecular Docking Simulation: In silico approach to study interaction between Ag/AgNPs and digestive enzymes in termite

Details about the interactions of proteins present in termite sp. participating in cellulose degradation with Ag and AgNPs were evaluated by molecular docking. Two enzymes were chosen according to their structure availability in Protein Data Bank (PDB) platform: i. the structure of Endoglucanase from termite, *Nasutitermes takasagoensis*, PDB:

1KS8, structure obtained by X-ray diffraction method at a resolution of 1.4 Å, a monomer with a sequence length of 433 amino acids (Khademi et al., 2002), ii. the crystal structure of a bacterial domain containing xylanase from termite gut, *Globitermes brachycerastes*, PDB: 4HU8, a crystallographic structure with a resolution of 2.0 Å and a sequence length of 456 amino acids (Han et al., 2013). The Ag was obtained from PubChem database CID:23954 uploaded in Avogadro 1.2.0 to generate coordinates for the Ag.pdb file (Hanwell et al., 2012). The AgNP structure required for protein-AgNPs interaction was previously developed and validated by Kyrychenko et al. (2015) and has been provided to us by Chandankere et al. (2020). Briefly, the AgNP is a truncated octahedron with a face-centered cubic (fcc) crystalline structure containing 3871 Ag atoms with a diameter of approximately 4.5 nm (Kyrychenko et al., 2015). Docking calculus of the Ag and AgNP was performed using PatchDock server Beta 1.3 version. This program is a geometry-based molecular docking algorithm, a good approach for molecular shape complementarity (Duhovny et al., 2002; Schneidman-Duhovny et al., 2005). Default options were adopted in the server platform for complex type and root mean square deviations (RMSD) of 4.0 Å for clustering and all figures were made using the VMD program (Humphrey et al., 1996).

2.9. Statistical Analysis

Data for termites and plant growth-related experiments were represented as mean ± standard error. Means were compared using analysis of variance (ANOVA) followed by Tukey's HSD test for plant data and t-test for termite data at the significant level of $P \leq 0.05$. All statistical analyses and graphs plotting were done using 'multcomp' and 'ggpubr' packages in R statistical software (version 3.6.3).

3. Results and discussion

3.1. PsAgNPs synthesis and characterization

The present study provides a simple and rapid method for biosynthesis of AgNPs from freshly collected leaves of *G. eriocarpum* (Fig. 1). The visible color change of the mixture from transparent to dark brown (Fig. 2a) indicates an efficacious synthesis of PsAgNPs due to the reduction of Ag⁺ to Ag⁰. Interestingly, the synthesis process was faster under constant shaking than a static condition. The AgNPs exhibit unique optical properties indicative of shape and size due to their absorption efficiency (Lee and Jun, 2019). In this study, the UV-visible spectrum is showing the presence of a strong surface plasmon resonance (SPR) peak at ~457 nm, which represents the formation of AgNPs (Fig. 2b). AgNPs have extraordinary efficiency of absorption and scattering that leads to the appearance of an SPR peak at a visible range of light due to the excitation of free electrons on the surface of AgNPs (Aherne et al., 2008). Further characterization by SEM and TEM analysis provided a better view of the shape and morphology of PsAgNPs (Fig. 2 c,d). The spherical morphology of PsAgNPs in the size range of 4 - 44.5 nm was observed (Fig. 2 d,e). Due to Mie scattering effect, the absorption spectra of the spherical nanoparticles display a single SPR peak whereas anisotropy in nanoparticles leads to the development of more than one SPR peaks (Sosa et al., 2003; Amendola et al., 2007). The data obtained by Uv-visible spectroscopy and SEM are in accordance with this theory and confirm the spherical morphology of PsAgNPs in this study which was also validated by TEM. Moreover, PsAgNPs were found to be well dispersed indicating efficient capping agent properties of *G. eriocarpum* biomolecules.

Furthermore, the degree of crystallinity of the PsAgNPs was revealed by XRD analysis. The XRD spectrum showed the presence of 4 broad Bragg reflection peaks at 2θ angles of 38.01°, 44.25°, 64.20°, and 77.80° which were assigned to (111), (200), (220), and (311) crystallographic planes of face-centered cubic (fcc) structures of AgNPs, respectively (Fig. 2f). This pattern is indicating the crystalline nature of PsAgNPs as reported in earlier studies (Gong et al., 2018; Femi-Adepoju et al., 2019;

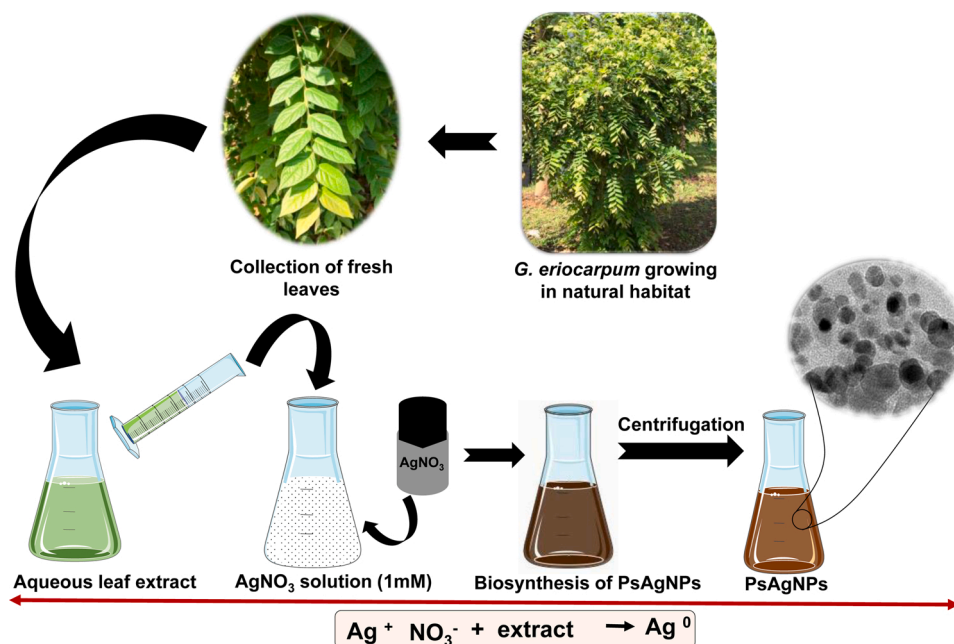


Fig. 1. Green synthesis route of PsAgNPs using aqueous leaf extract of *G. eriocarpum* growing in Xishuangbanna Tropical Botanical Garden.

Pirtarighat et al., 2019). Although the predominant crystalline phase was achieved due to silver, some unassigned peaks were also observed at $2\theta = 27.9^\circ$ and 32.05° that could be due to *G. eriocarpum* phytochemicals present in the leaf extract which represent bioorganic phase. This result is supported by previous findings (Jeeva et al., 2014; Behravan et al., 2019). Using the XRD spectrum, the average crystallite size was calculated to be 25.6 nm using Debye-Scherrer formula i.e. $D = k\lambda/\beta\cos\theta$, where, k is 0.9, β is full width at half maximum (FWHM) and θ is diffraction angle. The elemental characterization as detected by the EDAX spectrum indicated the purity of PsAgNPs. As shown in Fig. 2j, the intense peak at 3 keV indicated that silver (Ag) is the major element in the PsAgNPs represented by 84.4% weight. However, the other peaks of carbon (9.8% wt) and oxygen (5.8% wt) correspond to the biomolecules present in the leaf extract which are acting as capping agents. The occurrence of a strong peak at 3 keV is characteristic of a metallic silver due to surface plasmon resonance (Kaviya et al., 2011; Moodley et al., 2018; Dakshayani et al., 2019).

FTIR spectroscopy is an important analysis for characterizing the possible biomolecules aiding the reduction of silver ions and their further capping/stabilization. Functional groups representing the plant biomolecules associated with PsAgNPs can be identified using FTIR. Here, the FTIR spectrum is showing strong absorption bands at 3434, 2917, 1624, 1443, 1385, 1207, 1027, and 514 cm^{-1} . The peak at 3434 cm^{-1} corresponds to O–H stretching vibration arising due to the presence of alcohol and phenols. Another peak at 2917 cm^{-1} was assigned to C–H group of alkane/aromatic compounds. The band at $1624, 1443\text{ cm}^{-1}$ corresponds to C–N, C–C and N–H stretching vibrations present in proteins. The band at 1385 cm^{-1} arising due to N–O stretching of nitro compounds. The peaks near 1207 and 1027 cm^{-1} exemplify aliphatic C–N and carbonyl stretching in proteins. The band at 514 cm^{-1} is arising due to stretching vibration of alkynes (Fig. 2g). The obtained FTIR spectrum results correspond well with the previous studies on green synthesis of AgNPs using plants (Balashanmugam and Kalaichelvan, 2015; Femi-Adepoju et al., 2019; Pirtarighat et al., 2019). Additionally, the potential role of proteins present in *G. eriocarpum* leaf extracts for the biosynthesis of PsAgNPs was confirmed by the Raman spectrum showing a strong characteristic peak at 1599.4 cm^{-1} with the highest intensity (Fig. 2h). According to previous reports, the presence of 1600 cm^{-1} band resembles with benzene ring that could be possibly due to aromatic amino acids such as tyrosine, phenylalanine, tryptophan (Costa Silva et al., 2017;

Singh et al., 2017). As documented earlier, protein biomolecules in plant extract are excellent capping agents and lead to the stabilization of biosynthesized nanoparticles (de Barros et al., 2018; Pirtarighat et al., 2019). Altogether, the results indicate the effectiveness of phytochemicals of *G. eriocarpum* for the synthesis, capping, and stabilization of metal nanoparticles. Furthermore, TGA confirmed the thermal stability of PsAgNPs at a temperature range of $25\text{--}700^\circ\text{C}$. The first step minimal weight loss was recorded at $\sim 100\text{--}200^\circ\text{C}$. This weight loss was due to the removal of moisture adsorbed into PsAgNPs surface. The noticeable second step weight loss was recorded at a temperature range of $\sim 200\text{--}700^\circ\text{C}$. This weight loss is appearing due to the desorption of biomolecules such as carbohydrates, polyphenols, etc. (Sun et al., 2014; Moldovan et al., 2018) present on the surface of AgNPs. Most importantly, 82.85% residual weight was obtained after heating samples up to 700°C causing 17.15% weight loss that indicates significant thermal stability of the PsAgNPs (Fig. 2i). The high degree of thermal stability can be attributed to the significant role of protein biomolecules as capping agents of PsAgNPs as authenticated by FTIR and Raman spectroscopy. Based on these facts, we can say that *G. eriocarpum* is a suitable natural biosynthetic source for obtaining efficient AgNPs by following a simple synthesis process and without using hazardous or costly chemicals.

3.2. Effects of PsAgNPs on plant growth

Since the major anticipated areas for broad-range applications of PsAgNPs include agriculture and forestry therefore, we contemplated examining their impacts on plant growth in terms of seed germination and primary root growth. The PsAgNPs treatment did not cause an inhibitory impact on *Cajanus cajan* seeds germination. The pronounced toxic effect was observed for AgNO_3 treatment while seeds in both control and PsAgNPs treated set germinated well. A noticeable significant difference in the primary root and lateral root growth among the three treatments was observed (Fig. 3, Fig. S1). The primary root length in PsAgNPs (38.14 ± 1.36) and AgNO_3 (19.75 ± 1.97) treated set showed 35.44% increase and 29.86% decrease, respectively over the control set (28.16 ± 1.06) after 8 days of treatment (Fig. 3 a,b). Likewise, lateral root length showed a prominent significant difference among the three treatments but also showed a high range of variation compared to the primary root length. The lateral root length showed

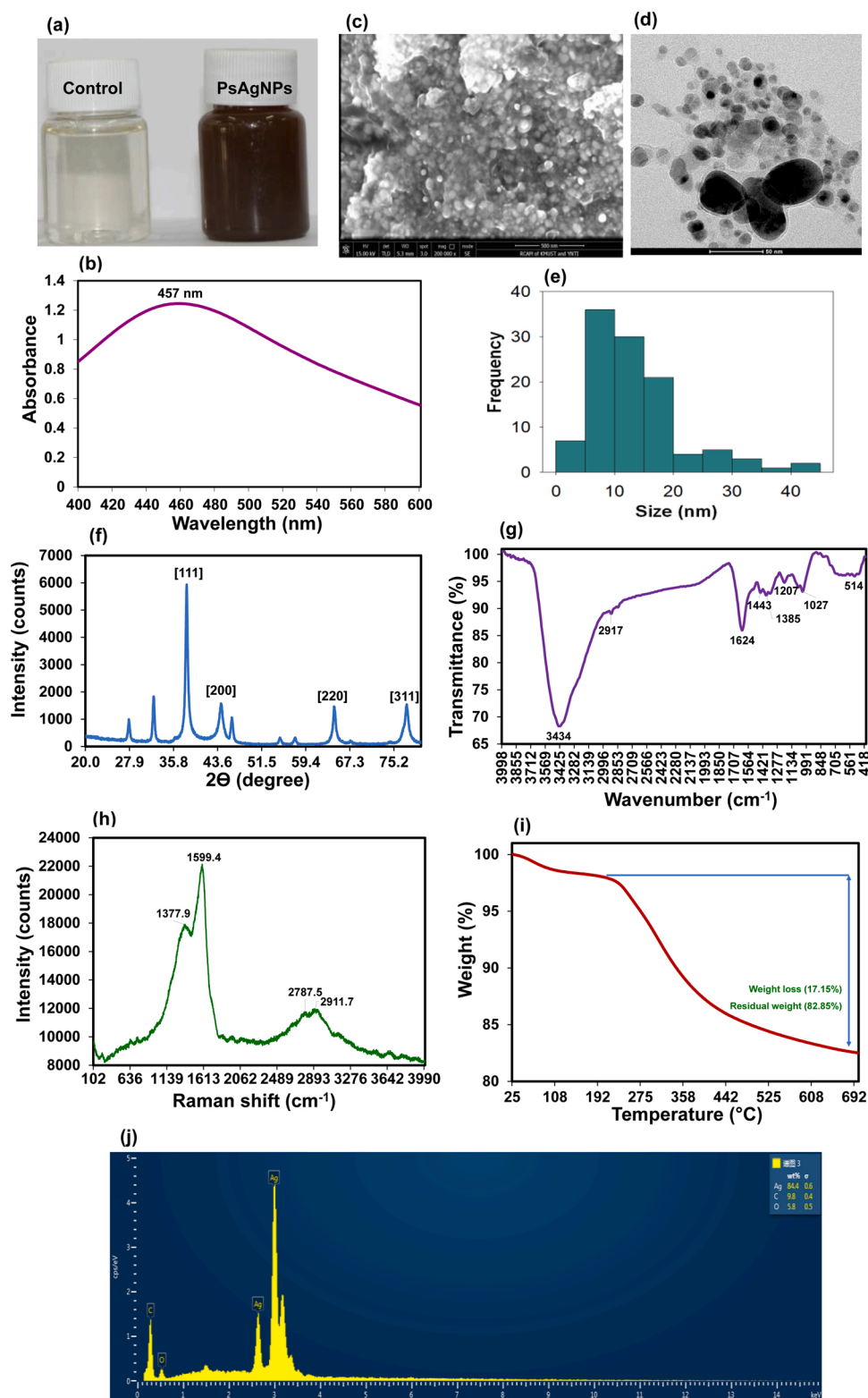


Fig. 2. Biosynthesis and characterization of PsAgNPs: (a) PsAgNPs showing visible color change after treatment with AgNO_3 ; (b) Uv-vis spectrum of PsAgNPs showing SPR peak at 457 nm; Morphological characterization of PsAgNPs using SEM (c) and TEM (d) microscopy; (e) size range distribution of PsAgNPs; XRD (f), FTIR (g) and Raman (h) spectra; (i) TGA showing thermal stability of PsAgNPs; (j) Elemental characterization: EDAX spectrum of PsAgNPs showing 84.4% Ag followed by C (9.8%) and O (5.8%).

33.53% decrease in AgNO_3 treatment (3.27 ± 0.60) and 35.56% increase due to PsAgNPs treatment (6.67 ± 0.53) as compared to control set (4.92 ± 0.43) (Fig. 3 c,d). This result is supported by previous studies which suggest certain mechanisms such as improved water uptake by seeds (Mahakham et al., 2017), enhanced photosynthesis process, and stimulation of nitrogen transport (Das et al., 2018) are responsible for biosynthesized AgNPs mediated augmentation of seed germination and

plant growth. However, we can witness both kinds of effects (beneficial or toxic) of AgNPs in literature and due to discrepancy in results, the mechanism of AgNPs causing either positive or negative effects are not very well understood.

Furthermore, the cross-section of the primary root treated with AgNO_3 showed significant changes in anatomy as compared to control and PsAgNPs treatment (Fig. S2). The outer epidermis layer was

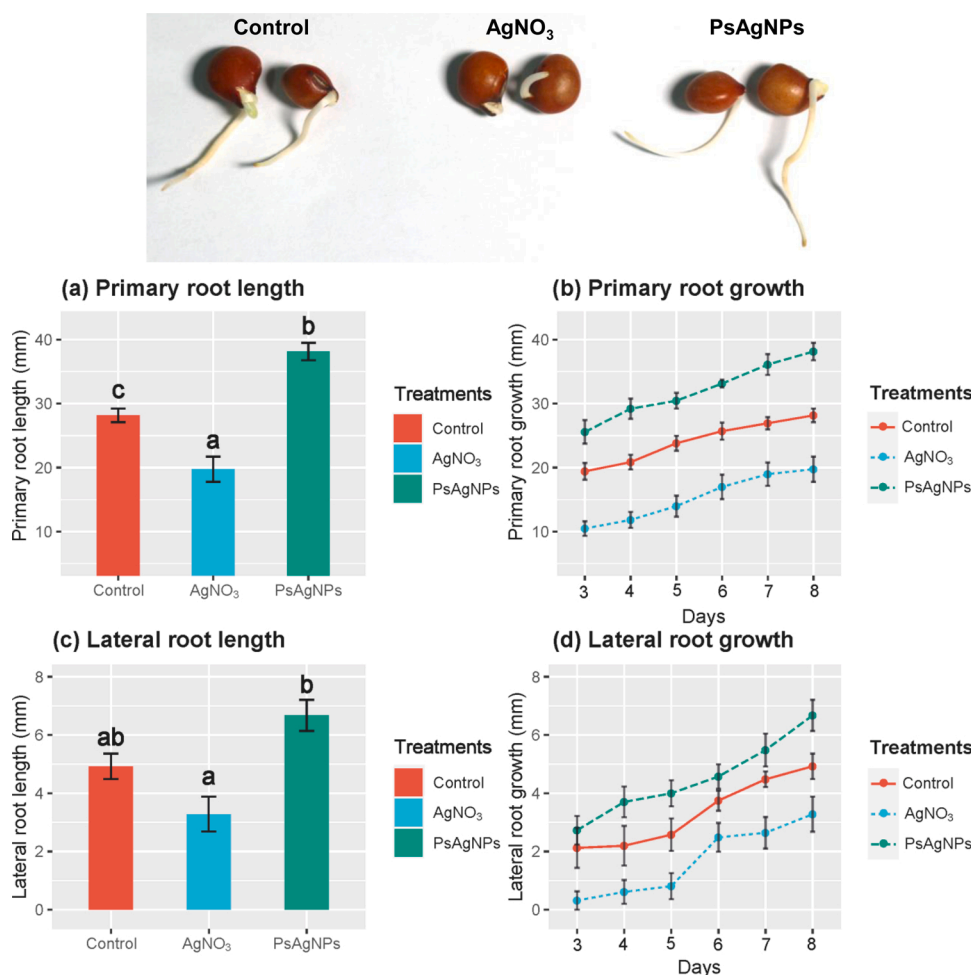


Fig. 3. Impact of PsAgNPs and AgNO₃ on *Cajanus cajan* seeds germination and primary root growth at different days. (a, c) Primary root length and lateral root length in different treatments after 8 days of treatments. Values for barplot represent mean \pm standard error ($n = 6$); different letters over the bar indicate that mean values of the three treatments are significantly different from each other as determined by one-way ANOVA followed by Tukey's post hoc test at $P < 0.05$. (b, d) Pattern of primary and lateral root growth among three treatments over the different time period.

observed to be thin and distorted in AgNO₃ treatment. Most importantly, a dark spot close to the vascular bundle was noticed. In contrast, no such irregularity and distortion of the epidermis was observed in control and PsAgNPs treatment. The presence of a dark spot could be possibly due to the enrichment of silver ions in cells that cause toxicity and further distortion of cells leading to reduced germination process. The bulk form of silver i.e. AgNO₃ can release silver ions after dissolution in water and then can easily be translocated through xylem vessels. We hypothesize that PsAgNPs, due to capping with *G. eriocarpum* biomolecules, are in more stabilized form and hence release of silver ions from PsAgNPs is not frequent. Furthermore, the sensitivity towards nanoparticles may vary for different plants and we assume that an optimum dose of PsAgNPs i.e. 50 $\mu\text{g/mL}$ is having growth stimulatory effects. The release of silver ions is the main toxicity determining factor (Liu et al., 2010; Reidy et al., 2013). Interestingly, AgNO₃ is reported to cause stronger phytotoxic effects than AgNPs due to the high accumulation of silver ions (Vishwakarma et al., 2017). Based on this, we assume that the dark spot close to the vascular bundle resulting from high silver accumulation in AgNO₃ treatment might be the possible mechanism of phytotoxicity. The high stabilization of PsAgNPs is another important clue as obtained by this data. We hypothesize that *G. eriocarpum* leaf extracts biomolecules are key players in determining the stability of PsAgNPs.

3.3. Evidence for pest controlling properties of PsAgNPs

The present study provides evidence for the novel future application of PsAgNPs based nanoformulation for pest management. Our rationale for this assumption is based on two key features: a) termites

(*Odontotermes* sp.) were collected from infested woods that indicate their wood-feeding activity, b) *Odontotermes* spp. have been identified as destructive pests of woods, trees, forest, crops (Chiu et al., 2018; Ahmad et al., 2019). Significant use of nanotechnology in the agricultural sector is gaining huge attention due to its multifarious applications (Mishra et al., 2017a, 2017b). Currently, Borges et al. (2018) proposed possibilities of implementing nanotechnology-based techniques for wood protection. In this regard, we suggest that PsAgNPs exhibiting excellent termite repellent activity could be used for termite pest management. Several studies are available showing antimicrobial properties of AgNPs (Sondi and Salopek-Sondi, 2004; Mishra et al., 2017b, 2014; Mishra and Singh, 2015; Loo et al., 2018), however, their activity against termite pests are largely missing and not well attempted. The choice-bioassay confirmed strong repellent activity of PsAgNPs compared to control ($t = 22.818$, $df = 11$, $P < 0.001$) (Fig. 4 a). The average number of individuals of termites making contact with the control part (50.83 ± 1.78) was always significantly higher than PsAgNPs treated part (5.41 ± 0.83). The repellent effect of PsAgNPs towards termites was found to be consistent each time during the repetition of the experiment showing a mean percent repellent efficiency of 80.97 ± 2.77 . Similarly, the anti-feedant effect of PsAgNPs (Fig. 4b) was also found to be promising in no-choice bioassay indicating antifeedant index (AI) of 53.49 ± 7.37 . Since the observation on feeding behavior in terms of paper consumption was made after one week therefore, we assume to get more AI value if we would have continued the experiment for 15–30 days. Tunneling activity is another important feature of pest termites as it allows them to reach the food source and hence affect their foraging efficiency (Xu et al., 2019). Therefore, understanding the tunneling behavior of

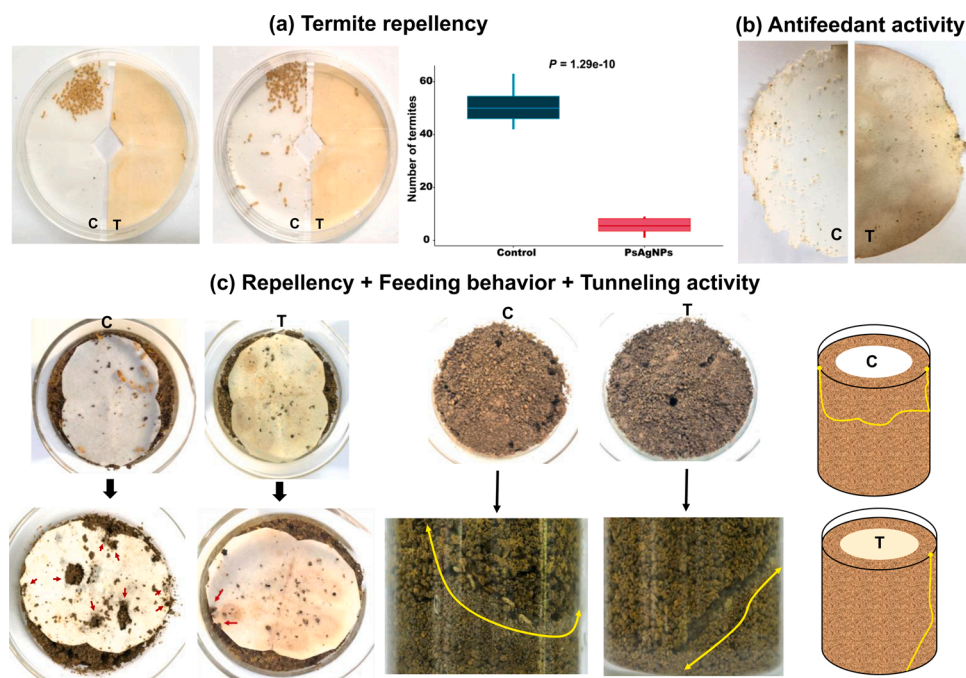


Fig. 4. Effect of PsAgNPs on different activities of termites. (a) Choice bioassay and boxplot showing termite repellent activity of PsAgNPs; data for boxplot have been compiled from 4 individual experimental setup (4 times \times 3 replicates; $n = 12$) repeated at random time for confirmation (b) Antifeedant activity displaying paper consumed in control and treated part (c) An experimental setup to observe repellency, feeding behavior and tunneling activity together. Red arrows indicate paper consumption and yellow arrows indicate path of tunnel constructed in experimental chambers. (C) control, (T) PsAgNPs treatment.

termites as influenced by PsAgNPs treatment can help us to develop more effective pest control measures. The most effective way for sustainable pest management could be achieved via targeting feeding behavior, tunneling activity, and repellency of termite pests. For that reason, we used an experimental setup to observe all the above activities together and to capture a more holistic view. As shown in Fig. 4c, we observed a noticeable difference in the tunneling pattern between control and PsAgNPs treated chamber. In the control set, tunneling activity was higher resulting in many interconnected tunnels at a height of 2.5 cm from the top. Tunnels were constructed in such a way that termites can easily reach and attack filter paper (on the top) as evident from paper consumption. Moreover, the movement of termites within visible tunnels was observed very frequently. In contrast, in the PsAgNPs treated chamber, termites adopted different tunneling strategy and they tended to avoid the treated filter paper disc. One very prominent visible tunnel going straight down to the bottom was observed and termites movement was also restricted possibly due to their avoidance strategy leading to initiate hiding.

Recent recognition of integration between nanotechnology and arthropod control offers a new area of research that extends our knowledge beyond the well-studied antimicrobial potential of a myriad of metal nanoparticles. This offers a potential way to control a range of pests, insects, vectors (Amerasan et al., 2016; Wakeil et al., 2017; Pascoli et al., 2019). Although previous efforts have been focused more on exploiting toxic effects of nanoparticles against mosquitoes, parasites and ticks (Abinaya et al., 2018; Alyahya et al., 2018; Amerasan et al., 2016; Benelli, 2018; Benelli, 2016), their efficacy against termites has been the least explored area. Dorau et al. (2004) proposed the idea to use silver-based biocides as wood protectants to restrict fungal decay and termite damage. They recommended advantages of a range of silver salts such as silver bromide, silver chloride, silver nitrate and silver thiosulfate over chromated copper arsenate, a widely used wood preservative for health and environment toxicity concerns. Likewise, Qi et al. (2011) also reported an important role of Copper-Carbon Core-Shell nanoparticles for forest protection considering its efficacy against termites and wood-decaying fungi. Our understandings of the potency of bio-synthesized AgNPs for maintaining forest health, forest trees growth promotion, wood protection is in infancy. It is obvious from previous reports that very limited efforts have been put to test the efficiency of

AgNPs (more specifically biosynthesized AgNPs) against termites, therefore there are significant knowledge gaps about the key role and probable mechanisms of action of AgNPs against termites. We argue that this study highlights a novel application of PsAgNPs against termites pointing towards their future application in the agriculture and forestry sectors for pest management. Nanotechnology holds promising applications in the forest (McCrack, 2009) and we need to discover the potency of synthesized metal nanoparticles for their successful applications in maintaining the forest health. The mechanisms of AgNPs toxicity to insects could be due to oxidative stress, DNA & organelles damage, membrane permeability disruption, and intracellular enzyme deactivation (Jiang et al., 2015; Benelli, 2018; Mao et al., 2018). More specifically, toxicity to insects is triggered mainly due to the release of silver ions. In addition to this, prior studies documented that AgNPs can cause up/downregulation of oxidative stress-related genes, downregulation of ribosomal protein causing protein synthesis inhibition, attenuation of signal transduction pathway, etc., leading to the development of toxicity behavior towards *Chironomus riparius*, Lepidopteran insects (Nair et al., 2011; Nair and Choi, 2012, 2011; Yasur and Usha Rani, 2015). In our study, contact toxicity assay provided useful information about PsAgNPs toxicity to termites. We observed some visible changes such as whole-body shrinkage, contracted abdomen, and abnormal head structure in termites exposed to PsAgNPs (Fig. 5). Interestingly, we noticed dark-colored lower abdomen (Fig. 5d, Supplementary data S3) which might be due to intake and accumulation of AgNPs that can damage their digestive systems. This observation could also be related to the absorption of nanoparticles by termites as PsAgNPs could also be seen attached to their body.

3.4. Molecular docking simulation for Protein-Ag and Protein-AgNPs interactions: Impact on digestive enzymes

Termites mainly feed on the primary nutrition source i.e. lignocellulose which is the major component of woody plants. The unique ability to digest lignocellulose is driven by a variety of enzymes secreted by termites host itself and gut-associated symbionts (Brune, 2014). Therefore, two different proteins were used because of their distinct origins: xylanase from *Globitermes brachycerastes* termite gut bacteria and endoglucanase from termite *Nasutitermes takasagoensis* (Khademi et al.,

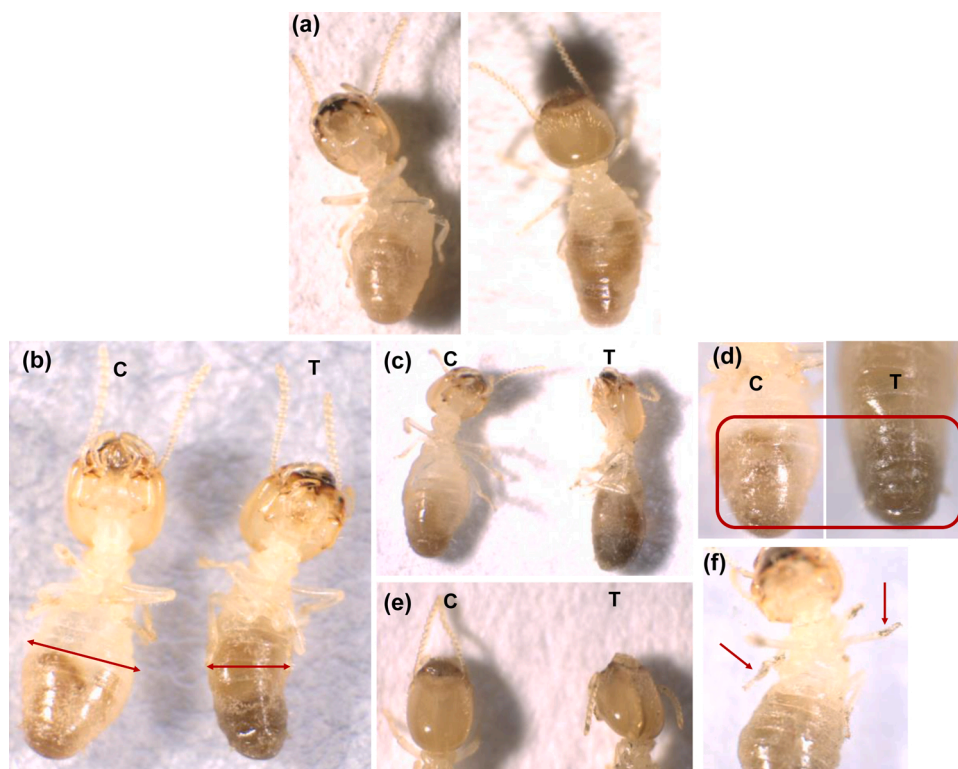


Fig. 5. No-choice bioassay: Pictures are showing visible morphological changes in termites after 24 h of exposure to PsAgNPs. (a) termites soon after collection and before exposure; (b) red arrow indicate the shrinkage of abdominal part after exposure; (c, d, e) visible changes in body parts such as head structure, dark colored lower abdomen and whole-body shrinkage; (f) PsAgNPs attached to legs. C (control) and T (PsAgNPs exposure).

2002; Han et al., 2013). Most importantly, we selected these two proteins structure from termites showing the same wood-feeding behavior. The objective was to evaluate the interactions of Ag and AgNPs with digestive enzymes of termites, considering the chemical element in its elementary and nanoparticle forms. Previously, Dorau et al. (2004) documented that silver ions released from silver salt can attack micro-organisms in the termite gut resulting in the impaired digestive system that causes toxic impacts on termites. In our contact toxicity assay, we observed dark-colored lower abdomen due to PsAgNPs exposure (Supplementary data S3). This observation provided us a practical clue for AgNPs mediated damage to the digestive system of termites. Hence, we attempted to provide evidence based on molecular docking results.

The endo- β -1,4-glucanases compose a group of endogenous cellulase present in termites responsible for degrading carboxymethyl cellulose (CMC), belonging to glycosyl hydrolase family 9. The majority of these proteins have a similar structure showing a catalytic domain linked to a cellulose-binding domain (CBD), except for plants and termites which contain a single catalytic site without CBD and linkers (Khademi et al., 2002). The xylanase adopted here was obtained from gut bacteria of a wood-feeding termite (*Globitermes brachycastrus*). This enzyme belongs to glycoside hydrolases (GH) family with common synonyms as endoxylanase or 1,4- β -D-xylanohydrolases, which catalyzes the hydrolysis of the internal 1,4- β -D-xylonic bond of xylan, the major component of hemicellulose in plant cell walls (Han et al., 2013).

Details about the Ag-endoglucanase and Ag-xylanase interactions are presented in Fig. 6. The endoglucanase enzyme has Glu412 as the catalytic acid/base residue and Asp54 or Asp57 as the basic residue. Moreover, this enzyme has an important binding site close to the catalytic pocket responsible for interacting with Ca^{2+} (Chauvaux et al., 1992; Khademi et al., 2002) as shown in Fig. 6a. Docking analysis showed that the protein surface can stabilize Ag at several cavities, mainly in its front-face, i.e., closest to the catalytic and Ca^{2+} -binding domains. Ag was found close to Asp54, Asp57, and Glu 412 in the catalytic site, and

also close to Asp210, Asp213, Glu214, and Asp254 in the calcium domain. In this regard, it is possible to suggest a disturbance promoted by Ag in these protein types, compromising the cellulose degradation in termite guts via competitive interaction with the substrate to the catalytic pocket. Moreover, Ca^{2+} -binding sites are important, for example, for endoglucanase from *C. thermocellum*, for stability and the kinetic properties of the enzyme (Chauvaux et al., 1995). Similarly, the GH xylanase can stabilize Ag in its surface, as depicted in Fig. 6b. This protein has two distinct domains, a catalytic domain – CD, and a non-catalytic domain. Interestingly, Ag was only docked in the CD domain, suggesting a specific contribution for disturbance in the catalytic reaction. The docking results pointed out some possibilities for mostly direct contributions for enzyme inhibition, according to Ag-protein interactions on to the catalytic site, specifically with Trp205, Glu251, Asn299, Gln341, His343, Glu371, Trp430, and Trp438. In general, both proteins can be disturbed by Ag in its elementary form due to the accommodation of Ag on the protein surface vacancies, affecting the kinetics and yield for cellulose degradations.

In the next step, we hypothesized that the most suitable chemical structure for understanding the inhibition process is AgNPs. Therefore, we also performed molecular docking to assess protein-AgNPs interactions. The results from Protein-AgNP interactions suggest an important contribution of silver nanoparticles in the inhibition process through partial blocking of the catalytic pocket entrance. For both enzymes, our findings might justify an avoided substrate accessibility leading to a disturbance in the catalytic reaction. In Fig. 7a, there is a unique position assumed by AgNPs for all 20 runs, suggesting its high stability in the catalytic and Ca^{2+} -binding domains. Hence, it is expected that AgNPs are stabilized in a critical surface region of the endoglucanase, especially on the calcium linkage domain. On the other hand, for xylanase, the best position was found in the “back-face” of the protein catalytic domain, 18 structures, and two structures closest to the CD surface, as detailed in Fig. 7b. Similar to that observed for Ag-xylanase

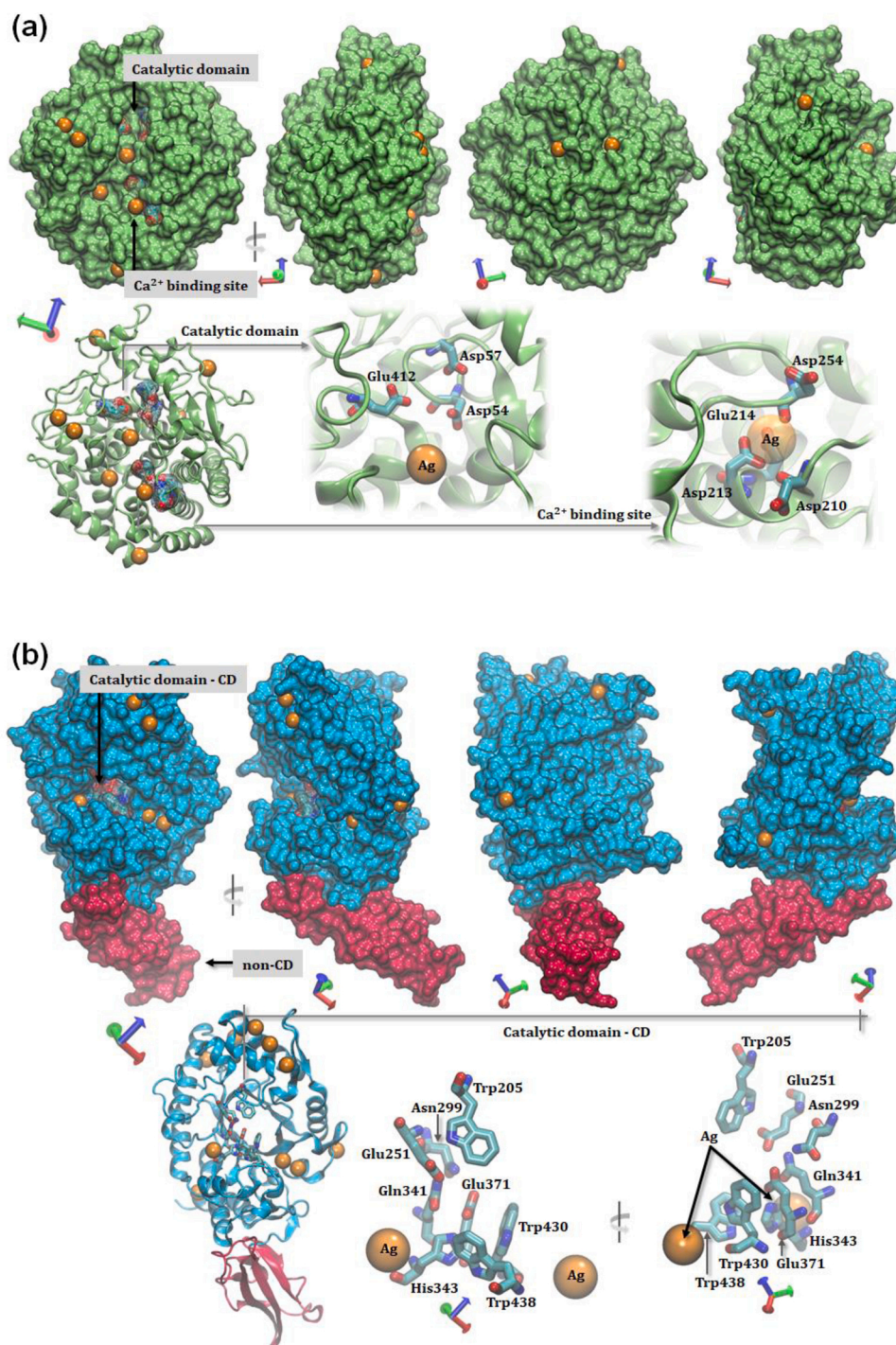


Fig. 6. Docking analysis of the Ag deposition at different parts of the protein surfaces. (a) Details for endoglucanase, PDB:1KS8, depicting the Ag deposition close to the catalytic and Ca²⁺-binding domains. (b) Interaction with xylanase, PDB:4HU8, highlighting the Ag interactions with residues that compose the catalytic site, such as Trp205, Glu251, Asn299, Gln341, His343, Glu371, Trp430 and Trp438. All positions assumed by Ag were the 10 best scored values according to the docking Patch-Dock program.

interactions (Fig. 6b), there was no deposition of AgNPs sampled in the non-CD region highlighting the possibility of nanoparticles acting directly in the catalytic process via steric disturbance of the substrate accessibility. Apart from this, indirect disturbance by nanoparticles stabilized far away from the catalytic site may also be considered. This behavior can promote changes in the protein structure, as already suggested, for instance, for thrombin protein (Chandankere et al., 2020).

4. Conclusions

In recent years, with the purpose of generating environment-friendly nanoparticles encompassing a vast range of applications, the green

synthesis approach involving plants has gained great attention. Nevertheless, embracing sustainability in a way that is beneficial to the environment and humankind is most important and required constantly. Hence, the increasing demand and usage of plants for industrial applications must be compensated with their cultivation on a large scale. Therefore, we propose that *G. eriocarpum* must be used prudently for the large-scale synthesis of AgNPs. Additionally, there is also need to identify potential biomolecules of *G. eriocarpum* facilitating synthesis of PsAgNPs as it would help augment the synthesis process and enhance properties and stability of generated nanoparticles.

Secondly, this preliminary study is intended to suggest probable future applications of AgNPs against potentially damaging termite pests

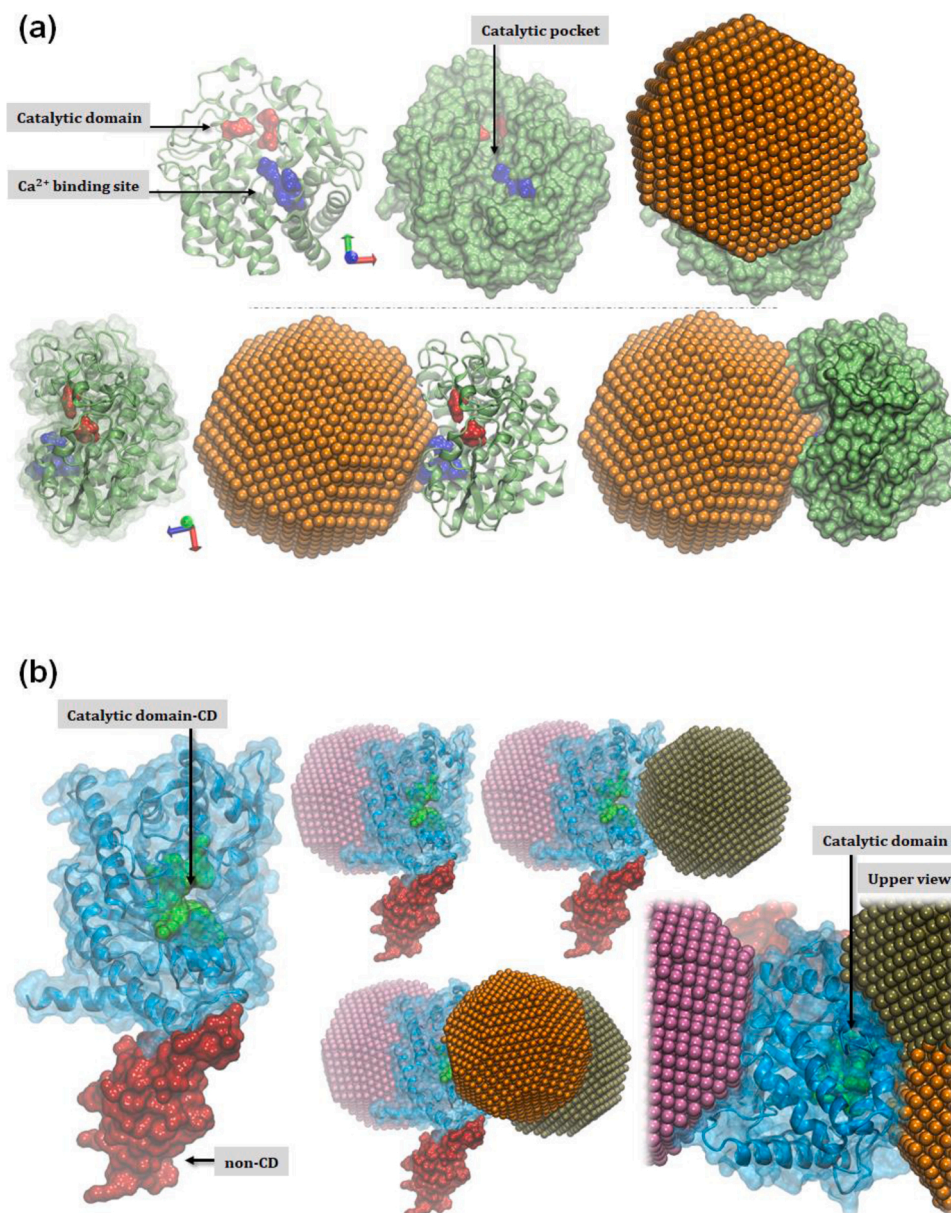


Fig. 7. Protein-AgNP interactions calculated by molecular docking. (a) Interaction sampled by endoglucanase, PDB:1KS8, and AgNPs representing the total of 20 runs, showing that nanoparticle is accommodated in the catalytic pocket entrance. (b) Disposition of AgNPs around the xylanase, PDB:4HU8, suggesting three distinct positions assumed by the nanoparticle. In both proteins, the catalytic domains are partially closed by AgNPs.

in agriculture and agroforestry sectors for pest management to prevent damages to living trees, woods, crops, etc. caused by termites. Moreover, incorporating this strategy as a part of an integrated pest management approach can be fruitful in reducing the excessive use of pesticides and minimizing their potential hazards to the environment. However, the third most important point for consideration and a future goal is to elucidate the environmental compatibility of biosynthesized PsAgNPs. Whilst the green synthesis approach is considered as environment-friendly, we recommend for more extensive research for understanding the all possible impacts of PsAgNPs on plants, soil, soil biota, beneficial microorganisms, and associated functions, etc. and to optimize the environmentally relevant dose to ensure their safe applications for environmental protection and achieving sustainability.

CRediT authorship contribution statement

Sandhya Mishra: Conceptualization, Methodology, Investigation,

Visualization, Data curation, Funding acquisition, Writing - original draft, Writing - review & editing. **Wenting Wang:** Investigation, Formal analysis. **Ivan Pires de Oliveira:** Software, Visualization, Data curation, Writing - review & editing. **Anjana J. Atapattu:** Data curation. **Shang-Wen Xia:** Writing - review & editing. **Renato Grillo:** Visualization, Writing - review & editing. **Caroline Honaier Lescano:** Software, Visualization, Writing - review & editing. **Xiaodong Yang:** Supervision, Conceptualization, Resources, Funding acquisition, Writing - review & editing, Project administration.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jhazmat.2020.123840>.

References

- Abinaya, M., Vaseeharan, B., Divya, M., Sharmili, A., Govindarajan, M., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M., Benelli, G., 2018. Bacterial exopolysaccharide (EPS)-coated ZnO nanoparticles showed high antibiofilm activity and larvicidal toxicity against malaria and Zika virus vectors. *J. Trace Elem. Med. Biol.* 45, 93–103. <https://doi.org/10.1016/j.jtemb.2017.10.002>.
- Aherne, D., Ledwith, D.M., Gara, M., Kelly, J.M., 2008. Optical properties and growth aspects of silver nanoprisms produced by a highly reproducible and rapid synthesis at room temperature. *Adv. Funct. Mater.* 18, 2005–2016. <https://doi.org/10.1002/adfm.200800233>.
- Ahmad, F., Fouad, H., Liang, S., Hu, Y., Mo, J., 2019. Termites and Chinese agricultural system: applications and advances in integrated termite management and chemical control. *Insect Sci.* 1744–7917, 12726. <https://doi.org/10.1111/1744-7917.12726>.
- Ahmed, S., Saifullah Ahmad, M., Swami, B.L., Ikram, S., 2016. Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. *J. Radiat. Res. Appl. Sci.* 9, 1–7. <https://doi.org/10.1016/j.jrras.2015.06.006>.
- Alyahya, S.A., Govindarajan, M., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M., Mothana, R.A., Al-anbr, M.N., Vaseeharan, B., Ishwarya, R., Yazhiniprabha, M., Benelli, G., 2018. Swift fabrication of Ag nanostructures using a colloidal solution of *Holostemma ada-kodien* (Apocynaceae) – Antibiofilm potential, insecticidal activity against mosquitoes and non-target impact on water bugs. *J. Photochem. Photobiol. B Biol.* 181, 70–79. <https://doi.org/10.1016/j.jphotobiol.2018.02.019>.
- Amendola, V., Polizzi, S., Meneghetti, M., 2007. Free silver nanoparticles synthesized by laser ablation in organic solvents and their easy functionalization. *Langmuir* 23, 6766–6770. <https://doi.org/10.1021/la0637061>.
- Amerasan, D., Nataraj, T., Murugan, K., Panneerselvam, C., Madhizhagan, P., Nicoletti, M., Benelli, G., 2016. Myco-synthesis of silver nanoparticles using *Metarhizium anisopliae* against the rural malaria vector *Anopheles culicifacies* Giles (Diptera: Culicidae). *J. Pest Sci.* 89 (2004), 249–256. <https://doi.org/10.1007/s10340-015-0675-x>.
- Arunachalam, R., Dhanasingh, S., Kalimuthu, B., Uthirappan, M., Rose, C., Mandal, A.B., 2012. Phytosynthesis of silver nanoparticles using *Coccinia grandis* leaf extract and its application in the photocatalytic degradation. *Colloids Surfaces B Biointerfaces* 94, 226–230. <https://doi.org/10.1016/j.colsurfb.2012.01.040>.
- Balashanmugam, P., Kalaichelvan, P.T., 2015. Biosynthesis characterization of silver nanoparticles using *Cassia roxburghii* DC. aqueous extract, and coated on cotton cloth for effective antibacterial activity. *Int. J. Nanomedicine* 87. <https://doi.org/10.2147/IJN.S79984>.
- Behravan, M., Hossein Panahi, A., Naghizadeh, A., Ziaee, M., Mahdavi, R., Mirzapor, A., 2019. Facile green synthesis of silver nanoparticles using *Berberis vulgaris* leaf and root aqueous extract and its antibacterial activity. *Int. J. Biol. Macromol.* 124, 148–154. <https://doi.org/10.1016/j.jbiomac.2018.11.101>.
- Benelli, G., 2018. Mode of action of nanoparticles against insects. *Environ. Sci. Pollut. Res.* 25, 12329–12341. <https://doi.org/10.1007/s11356-018-1850-4>.
- Benelli, G., 2016. Green synthesized nanoparticles in the fight against mosquito-borne diseases and cancer—a brief review. *Enzyme Microb. Technol.* 95, 58–68. <https://doi.org/10.1016/j.enzmictec.2016.08.022>.
- Bignell, D.E., 2006. Termites as soil engineers and soil processors. *Intestinal Microorganisms of Termites and Other Invertebrates*. Springer, Berlin, Heidelberg, pp. 183–220.
- Borges, C.C., Tonoli, G.H.D., Cruz, T.M., Duarte, P.J., Junqueira, T.A., 2018. Nanoparticles-based wood preservatives: the next generation of wood protection? *CERNE* 24, 397–407. <https://doi.org/10.1590/01047760201824042531>.
- Brune, A., 2014. Symbiotic digestion of lignocellulose in termite guts. *Nat. Rev. Microbiol.* 12, 168–180. <https://doi.org/10.1038/nrmicro3182>.
- Burduşel, A.-C., Gherasim, O., Grumeşescu, A.M., Mogoantă, L., Ficai, A., Andronescu, E., 2018. Biomedical Applications of Silver Nanoparticles: An Up-to-Date Overview. *Nanomaterials* 8, 681. <https://doi.org/10.3390/nano8090681>.
- Chandankere, R., Chelliah, J., Subban, K., Shanadrahalli, V.C., Parvez, A., Zaved, H.M., Sharma, Y.C., Qi, X., 2020. Pleiotropic functions and biological potentials of silver nanoparticles synthesized by an endophytic fungus. *Front. Bioeng. Biotechnol.* 8. <https://doi.org/10.3389/fbioe.2020.00095>.
- Chauvaux, S., Beguin, P., Aubert, J.P., 1992. Site-directed mutagenesis of essential carboxylic residues in *Clostridium thermocellum* endoglucanase CelD. *J. Biol. Chem.* 267, 9757–9762. <https://doi.org/10.1074/jbc.270.17.9757>.
- Chiu, C.-I., Yeh, H.-T., Li, P.-L., Kuo, C.-Y., Tsai, M.-J., Li, H.-F., 2018. Foraging phenology of the fungus-growing termite *Odontotermes formosanus* (Blattodea: Termitidae). *Environ. Entomol.* 47 (6), 1509–1516. <https://doi.org/10.1093/ee/nvy140>.
- Costa Silva, L.P., Pinto Oliveira, J., Keijok, W.J., da Silva, A.R., Aguiar, A.R., Guimarães, M.C.C., Ferraz, C.M., Araújo, J.V., Tobias, F.L., Braga, F.R., 2017. Extracellular biosynthesis of silver nanoparticles using the cell-free filtrate of nematophagous fungus *Duddingtonia flagrans*. *Int. J. Nanomedicine Volume* 12, 6373–6381. <https://doi.org/10.2147/IJN.S137703>.
- Dakshayani, S.S., Marulasiddeshwara, M.B., Kumar, S., Golla, R., Devaraja, S.R.H.K., Hosamani, R., 2019. Antimicrobial, anticoagulant and antiplatelet activities of green synthesized silver nanoparticles using *Selaginella* (Sanjeevini) plant extract. *Int. J. Biol. Macromol.* 131, 787–797. <https://doi.org/10.1016/j.jbiomac.2019.01.222>.
- Das, P., Barua, S., Sarkar, S., Karak, N., Bhattacharyya, P., Raza, N., Kim, K.-H., Bhattacharya, S.S., 2018. Plant extract-mediated green silver nanoparticles: Efficacy as soil conditioner and plant growth promoter. *J. Hazard. Mater.* 346, 62–72. <https://doi.org/10.1016/j.jhazmat.2017.12.020>.
- de Barros, C.H.N., Cruz, G., Mayrink, W., Tasic, L., 2018. Bio-based synthesis of silver nanoparticles from orange waste: effects of distinct biomolecule coatings on size, morphology, and antimicrobial activity. *Nanotechnol. Sci. Appl.* 11, 1–14. <https://doi.org/10.2147/NSA.S156115>.
- Dorai, B., Arango, R., Green, F., 2004. An investigation into the potential of ionic silver as a wood preservative. *Proceedings from the Woodframe Housing Durability and Disaster Issues Conference* 133–145.
- Duhovny, D., Nussinov, R., Wolfson, H.J., 2002. Efficient Unbound Docking of Rigid Molecules. *Lecture Notes in Computer Science (Including Subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)* 185–200. https://doi.org/10.1007/3-540-45784-4_14.
- Femi-Adepoju, A.G., Dada, A.O., Otun, K.O., Adepoju, A.O., Fatoba, O.P., 2019. Green synthesis of silver nanoparticles using terrestrial fern (*Gleichenia pectinata* (Willd.) C. Presl.): characterization and antimicrobial studies. *Heliyon* 5, e01543. <https://doi.org/10.1016/j.heliyon.2019.e01543>.
- Gong, C.-P., Li, S.-C., Wang, R.-Y., 2018. Development of biosynthesized silver nanoparticles based formulation for treating wounds during nursing care in hospitals. *J. Photochem. Photobiol. B Biol.* 183, 137–141. <https://doi.org/10.1016/j.jphotobiol.2018.04.030>.
- Gopinath, K., Kumaraguru, S., Bhakharaj, K., Mohan, S., Venkatesh, K.S., Esakirajan, M., Kaleeswarran, P., Alharbi, N.S., Kadaikunnan, S., Govindarajan, M., Benelli, G., Arumugam, A., 2016. Green synthesis of silver, gold and silver/gold bimetallic nanoparticles using the *Gloriosa superba* leaf extract and their antibacterial and antibiofilm activities. *Microb. Pathog.* 101, 1–11. <https://doi.org/10.1016/j.micpath.2016.10.011>.
- Gopinath, V., Mubarakali, D., Priyadarshini, S., Priyadarshini, N.M., Thajuddin, N., Velusamy, P., 2012. Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: A novel biological approach. *Colloids Surfaces B Biointerfaces* 96, 69–74. <https://doi.org/10.1016/j.colsurfb.2012.03.023>.
- Goswami, A., Roy, I., Sengupta, S., Debnath, N., 2010. Novel applications of solid and liquid formulations of nanoparticles against insect pests and pathogens. *Thin Solid Films* 519, 1252–1257. <https://doi.org/10.1016/j.tsf.2010.08.079>.
- Govorushko, S., 2019. Economic and ecological importance of termites: A global review. *Entomol. Sci.* 22, 21–35. <https://doi.org/10.1111/ens.12328>.
- Han, Q., Liu, N., Robinson, H., Cao, L., Qian, C., Wang, Qianfu, Xie, L., Ding, H., Wang, Qian, Huang, Y., Li, J., Zhou, Z., 2013. Biochemical characterization and crystal structure of a GH10 xylanase from termite gut bacteria reveal a novel structural feature and significance of its bacterial Ig-like domain. *Biotechnol. Bioeng.* 110, 3093–3103. <https://doi.org/10.1002/bit.24982>.
- Hanwell, M.D., Curtis, D.E., Lonie, D.C., Vandermeersch, T., Zurek, E., Hutchison, G.R., 2012. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *J. Cheminform.* 4, 17. <https://doi.org/10.1186/1758-2946-4-17>.
- Humphrey, W., Dalke, A., Schulten, K., 1996. VMD: Visual molecular dynamics. *J. Mol. Graph.* 14, 33–38. [https://doi.org/10.1016/0263-7855\(96\)00018-5](https://doi.org/10.1016/0263-7855(96)00018-5).
- Iravani, S., 2011. Green synthesis of metal nanoparticles using plants. *Green Chem.* 13, 2638. <https://doi.org/10.1039/c1gc15386b>.
- Jain, S., Mehata, M.S., 2017. Medicinal Plant leaf extract and pure flavonoid mediated green synthesis of silver nanoparticles and their enhanced antibacterial property. *Sci. Rep.* 7, 15867. <https://doi.org/10.1038/s41598-017-15724-8>.
- Jeeva, K., Thiagarajan, M., Elangovan, V., Geetha, N., Venkatchalam, P., 2014. *Caesalpinia coriaria* leaf extracts mediated biosynthesis of metallic silver nanoparticles and their antibacterial activity against clinically isolated pathogens. *Ind. Crops Prod.* 52, 714–720. <https://doi.org/10.1016/j.indcrop.2013.11.037>.
- Jiang, X., Miclaus, T., Wang, L., Foldbjerg, R., Sutherland, D.S., Autrup, H., Chen, C., Beer, C., 2015. Fast intracellular dissolution and persistent cellular uptake of silver nanoparticles in CHO-K1 cells: implication for cytotoxicity. *Nanotoxicology* 9, 181–189. <https://doi.org/10.3109/17435390.2014.907457>.

- Jouquet, P., Traoré, S., Choosai, C., Hartmann, C., Bignell, D., 2011. Influence of termites on ecosystem functioning. Ecosystem services provided by termites. *Eur. J. Soil Biol.* 47, 215–222. <https://doi.org/10.1016/j.ejsobi.2011.05.005>.
- Junqueira, L.K., Florencio, D.F., 2018. Termite damage in agriculture areas and implanted forests: An ecological approach, termites and sustainable management. Springer International Publishing, Cham. <https://doi.org/10.1007/978-3-319-68726-1>.
- Kaviya, S., Santhanalakshmi, J., Viswanathan, B., Muthumary, J., Srinivasan, K., 2011. Biosynthesis of silver nanoparticles using *Citrus sinensis* peel extract and its antibacterial activity. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* 79, 594–598. <https://doi.org/10.1016/j.saa.2011.03.040>.
- Khademi, S., Guarino, L.A., Watanabe, H., Tokuda, G., Meyer, E.F., 2002. Structure of an endoglucanase from termite, *Nasutitermes takasagoensis*. *Acta Crystallogr. Sect. D Biol. Crystallogr.* 58, 653–659. <https://doi.org/10.1107/S0907444902002366>.
- Krishnaraj, C., Jagan, E.G., Rajasekar, S., Selvakumar, P., Kalaichelvan, P.T., Mohan, N., 2010. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. *Colloids Surfaces B Biointerfaces* 76, 50–56. <https://doi.org/10.1016/j.colsurfb.2009.10.008>.
- Krithiga, N., Rajalakshmi, A., Jayachitra, A., 2015. Green Synthesis of Silver nanoparticles using leaf extracts of *Clitoria ternatea* and *Solanum nigrum* and study of its antibacterial effect against common nosocomial pathogens. *J. Nanosci.* 2015, 1–8. <https://doi.org/10.1155/2015/928204>.
- Kyrychenko, A., Korsun, O.M., Gubin, I.I., Kovalenko, S.M., Kalugin, O.N., 2015. Atomistic simulations of coating of silver nanoparticles with poly(vinylpyrrolidone) oligomers: Effect of oligomer chain length. *J. Phys. Chem. C* 119, 7888–7899. <https://doi.org/10.1021/jp510369a>.
- Lee, S., Jun, B.-H., 2019. Silver nanoparticles: Synthesis and application for nanomedicine. *Int. J. Mol. Sci.* 20, 865. <https://doi.org/10.3390/ijms20040865>.
- Li, X., Xu, H., Chen, Z.-S., Chen, G., 2011. Biosynthesis of nanoparticles by microorganisms and their applications. *J. Nanomater.* 2011, 1–16. <https://doi.org/10.1155/2011/270974>.
- Lima, T., de A., Pontual, E.V., Dornelles, L.P., Amorim, P.K., Sá, R.A., Coelho, L.C.B.B., Napoleão, T.H., Paiva, P.M.G., 2014. Digestive enzymes from workers and soldiers of termite *Nasutitermes corniger*. *Comp. Biochem. Physiol. Part B Biochem. Mol. Biol.* 176, 1–8. <https://doi.org/10.1016/j.cbpb.2014.07.001>.
- Liu, J., Sonshine, D.A., Shervani, S., Hurt, R.H., 2010. Controlled release of biologically active silver from nanosilver surfaces. *ACS Nano* 4, 6903–6913. <https://doi.org/10.1021/nn102272n>.
- Liu, S., Lin, X., Behm, J.E., Yuan, H., Stiblik, P., Šobotník, J., Gan, J., Xia, S., Yang, X., 2019. Comparative responses of termite functional and taxonomic diversity to land-use change. *Ecol. Entomol.* 44, 762–770. <https://doi.org/10.1111/een.12755>.
- Loo, Y.Y., Rukayadi, Y., Nor-Khaizura, M.-A.-R., Kuan, C.H., Chieng, B.W., Nishibuchi, M., Radu, S., 2018. In vitro antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens. *Front. Microbiol.* 9 <https://doi.org/10.3389/fmicb.2018.01555>.
- Mahakham, W., Sarmah, A.K., Maensiri, S., Theerakulpisut, P., 2017. Nanoprimer technology for enhancing germination and starch metabolism of aged rice seeds using phyto-synthesized silver nanoparticles. *Sci. Rep.* 7, 8263. <https://doi.org/10.1038/s41598-017-08669-5>.
- Mao, B.-H., Chen, Z.-Y., Wang, Y.-J., Yan, S.-J., 2018. Silver nanoparticles have lethal and sublethal adverse effects on development and longevity by inducing ROS-mediated stress responses. *Sci. Rep.* 8, 2445. <https://doi.org/10.1038/s41598-018-20728-z>.
- Marslin, G., Siram, K., Maqbool, Q., Selvakesavan, R., Kruszka, D., Kachlicki, P., Franklin, G., 2018. Secondary metabolites in the green synthesis of metallic nanoparticles. *Materials (Basel)* 11, 940. <https://doi.org/10.3390/ma11060940>.
- McCrack, J., 2009. Nanotechnology applications in the forest sector. *Nat. Resour. Canada*.
- Mishra, S., Keswani, C., Abhilash, P.C., Fraceto, L.F., Singh, H.B., 2017a. Integrated approach of agri-nanotechnology: Challenges and future trends. *Front. Plant Sci.* 8 <https://doi.org/10.3389/fpls.2017.00471>.
- Mishra, S., Singh, B.R., Naqvi, A.H., Singh, H.B., 2017b. Potential of biosynthesized silver nanoparticles using *Stenotrophomonas* sp. BHU-S7 (MTCC 5978) for management of soil-borne and foliar phytopathogens. *Sci. Rep.* 7, 45154. <https://doi.org/10.1038/srep45154>.
- Mishra, S., Singh, B.R., Singh, A., Keswani, C., Naqvi, A.H., Singh, H.B., 2014. Biofabricated silver nanoparticles act as a strong fungicide against *Bipolaris sorokiniana* causing spot blotch disease in wheat. *PLoS One* 9, e97881. <https://doi.org/10.1371/journal.pone.0097881>.
- Mishra, S., Singh, H.B., 2015. Biosynthesized silver nanoparticles as a nanoweapon against phytopathogens: exploring their scope and potential in agriculture. *Appl. Microbiol. Biotechnol.* 99, 1097–1107. <https://doi.org/10.1007/s00253-014-6296-0>.
- Moldovan, B., Sincari, V., Perde-Schrepler, M., David, L., 2018. Biosynthesis of silver nanoparticles using *Ligustrum Ovalifolium* fruits and their cytotoxic effect. *Nanomaterials* 8, 627. <https://doi.org/10.3390/nano8080627>.
- Moodley, J.S., Krishna, S.B.N., Pillay, K., Sershen Govender, P., 2018. Green synthesis of silver nanoparticles from *Moringa oleifera* leaf extracts and its antimicrobial potential. *Adv. Nat. Sci. Nanosci. Nanotechnol.* 9, 015011 <https://doi.org/10.1088/2043-6254/aaab22>.
- Mukunthan, K., Elumalai, E., Patel, T.N., Murty, V.R., 2011. *Catharanthus roseus*: a natural source for the synthesis of silver nanoparticles. *Asian Pac. J. Trop. Biomed.* 1, 270–274. [https://doi.org/10.1016/S2221-1691\(11\)60041-5](https://doi.org/10.1016/S2221-1691(11)60041-5).
- Nair, P.M.G., Choi, J., 2012. Modulation in the mRNA expression of ecdysone receptor gene in aquatic midge, *Chironomus riparius* upon exposure to nonylphenol and silver nanoparticles. *Environ. Toxicol. Pharmacol.* 33, 98–106. <https://doi.org/10.1016/j.etap.2011.09.006>.
- Nair, P.M.G., Choi, J., 2011. Identification, characterization and expression profiles of *Chironomus riparius* glutathione S-transferase (GST) genes in response to cadmium and silver nanoparticles exposure. *Aquat. Toxicol.* 101, 550–560. <https://doi.org/10.1016/j.aquatox.2010.12.006>.
- Nair, P.M.G., Park, S.Y., Lee, S.-W., Choi, J., 2011. Differential expression of ribosomal protein gene, gonadotrophin releasing hormone gene and Balbiani ring protein gene in silver nanoparticles exposed *Chironomus riparius*. *Aquat. Toxicol.* 101, 31–37. <https://doi.org/10.1016/j.aquatox.2010.08.013>.
- Narayanan, K.B., Sakthivel, N., 2010. Biological synthesis of metal nanoparticles by microbes. *Adv. Colloid Interface Sci.* 156, 1–13. <https://doi.org/10.1016/j.cis.2010.02.001>.
- Park, Y., Hong, Y.N., Weyers, A., Kim, Y.S., Linhardt, R.J., 2011. Polysaccharides and phytochemicals: a natural reservoir for the green synthesis of gold and silver nanoparticles. *IET Nanobiotechnology* 5, 69. <https://doi.org/10.1049/iet-nbt.2010.0033>.
- Pascoli, M., Jacques, M.T., Agarrayua, D.A., Avila, D.S., Lima, R., Fraceto, L.F., 2019. Neem oil based nanopesticide as an environmentally-friendly formulation for applications in sustainable agriculture: An ecotoxicological perspective. *Sci. Total Environ.* 677, 57–67. <https://doi.org/10.1016/j.scitotenv.2019.04.345>.
- Philip, D., Unni, C., Aromal, S.A., Vidhu, V.K., 2011. *Murraya Koenigii* leaf-assisted rapid green synthesis of silver and gold nanoparticles. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* 78, 899–904. <https://doi.org/10.1016/j.saa.2010.12.060>.
- Pirtirigat, S., Ghannadnia, M., Baghsahi, S., 2019. Green synthesis of silver nanoparticles using the plant extract of *Salvia spinosa* grown in vitro and their antibacterial activity assessment. *J. Nanostructure Chem.* 9, 1–9. <https://doi.org/10.1007/s40097-018-0291-4>.
- Qi, Y., Lian, K., Wu, Q., Li, Y., Danzy, M., Menard, R., Chin, K.L., Collins, D., Oliveria, F., Klepzig, K., 2011. Potentials of nanotechnology application in forest protection. TAPPI International Conference on Nanotechnology for Renewable Materials.
- Rai, M., Yadav, A., Gade, A., 2009. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol. Adv.* 27, 76–83. <https://doi.org/10.1016/j.biotechadv.2008.09.002>.
- Rech-Cainelli, V., Monteiro de Barros, N., Garcia-Gianni, S., Sbeghen-Loss, A.C., Heinzen, H., Ruiz Diaz, A., Migues, I., Specht, A., Cesio, M.V., 2015. Antifeedant and repellent effects of neotropical *Solanum* extracts on drywood termites, (*Cryptotermes brevis* Walker, Isoptera: Kalotermitidae). *Sociobiology* 62. <https://doi.org/10.13102/sociobiology.v62i1.82-87>.
- Reidy, B., Haase, A., Luch, A., Dawson, K., Lynch, I., 2013. Mechanisms of silver nanoparticle release, transformation and toxicity: A critical review of current knowledge and recommendations for future studies and applications. *Materials (Basel)* 6, 2295–2350. <https://doi.org/10.3390/ma6062295>.
- Schneidman-Duhovny, D., Inbar, Y., Nussinov, R., Wolfson, H.J., 2005. PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucleic Acids Res.* 33, W363–W367. <https://doi.org/10.1093/nar/gki481>.
- Singh, A.K., Prakash, P., Singh, R., Nandy, N., Firdaus, Z., Bansal, M., Singh, Ranjan K., Srivastava, A., Roy, J.K., Mishra, B., Singh, Rakesh K., 2017. *Curcumin* quantum dots mediated degradation of bacterial biofilms. *Front. Microbiol.* 8 <https://doi.org/10.3389/fmicb.2017.01517>.
- Singh, J., Dutta, T., Kim, K.-H., Rawat, M., Samddar, P., Kumar, P., 2018. Green synthesis of metals and their oxide nanoparticles: applications for environmental remediation. *J. Nanobiotechnology* 16, 84. <https://doi.org/10.1186/s12951-018-0408-4>.
- Sondi, I., Salopek-Sondi, B., 2004. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J. Colloid Interface Sci.* 275, 177–182. <https://doi.org/10.1016/j.jcis.2004.02.012>.
- Sosa, I.O., Noguez, C., Barrera, R.G., 2003. Optical properties of metal nanoparticles with arbitrary shapes. *J. Phys. Chem. B* 107, 6269–6275. <https://doi.org/10.1021/jp0274076>.
- Sun, Q., Cai, X., Li, J., Zheng, M., Chen, Z., Yu, C.-P., 2014. Green synthesis of silver nanoparticles using tea leaf extract and evaluation of their stability and antibacterial activity. *Colloids Surfaces A Physicochem. Eng. Asp.* 444, 226–231. <https://doi.org/10.1016/j.colsurfa.2013.12.065>.
- Tippayawat, P., Phromviyo, N., Boueroy, P., Chompoosor, A., 2016. Green synthesis of silver nanoparticles in aloe vera plant extract prepared by a hydrothermal method and their synergistic antibacterial activity. *PeerJ* 4, e2589. <https://doi.org/10.7717/peerj.2589>.
- Tripathi, A., Liu, S., Singh, P.K., Kumar, N., Pandey, A.C., Tripathi, D.K., Chauhan, D.K., Sahi, S., 2017. Differential phytotoxic responses of silver nitrate (AgNO₃) and silver nanoparticle (AgNps) in *Cucumis sativus* L. *Plant Gene* 11, 255–264. <https://doi.org/10.1016/j.plgene.2017.07.005>.
- Vishwakarma, K., Shweta Upadhyay, N., Singh, J., Liu, S., Singh, V.P., Prasad, S.M., Chauhan, D.K., Tripathi, D.K., Sharma, S., 2017. Differential phytotoxic impact of plant mediated silver nanoparticles (AgNPs) and silver nitrate (AgNO₃) on *Brassica* sp. *Front. Plant Sci.* 8 <https://doi.org/10.3389/fpls.2017.01501>.
- Wakeil, N., El Alkahtani, S., Gaafar, N., 2017. Is nanotechnology a promising field for insect pest control in IPM programs? New Pesticides and Soil Sensors. Elsevier, pp. 273–309. <https://doi.org/10.1016/B978-0-12-804299-1.00008-4>.
- Worrall, E., Hamid, A., Mody, K., Mitter, N., Pappu, H., 2018. Nanotechnology for Plant Disease Management. *Agronomy* 8, 285. <https://doi.org/10.3390/agronomy8120285>.
- Xu, H., Zhu, Q., Hu, W., Kim, K., Lei, C., Xiang, Y., Huang, Q., 2019. Impacts of different environmental factors on tunnelling behaviour of the subterranean termite *Odontotermes formosanus* (Shiraki). *Ethol. Ecol. Evol.* 31, 231–239. <https://doi.org/10.1080/03949370.2018.1561524>.

- Yao, G., Cai, J., Wu, Y., Yang, X., Oo, T.N., Moe, A.Z., Luo, S., 2018. Taxonomic studies of *Glochidion* (Phyllanthaceae) from the Indo-China Peninsula (I): *G. shanense*, a new species from Myanmar. *PhytoKeys* 96, 57–62. <https://doi.org/10.3897/phytokeys.96.24497>.
- Yasur, J., Usha Rani, P., 2015. Lepidopteran insect susceptibility to silver nanoparticles and measurement of changes in their growth, development and physiology. *Chemosphere* 124, 92–102. <https://doi.org/10.1016/j.chemosphere.2014.11.029>.
- Yu, C., Tang, J., Liu, X., Ren, X., Zhen, M., Wang, L., 2019. Green biosynthesis of silver nanoparticles using *Eriobotrya japonica* (Thunb.) leaf extract for reductive catalysis. *Materials* (Basel). 12, 189. <https://doi.org/10.3390/ma12010189>.
- Zhang, B., Liu, S., Lei, Q., Zhou, J., Long, C., 2020. Phytochemical constituents and pharmacological activities of a traditional medicinal plant, *Glochidion eriocarpum* (Phyllanthaceae), in: *Medicinal Plants: Biodiversity, Sustainable Utilization and Conservation*. Springer, Singapore, Singapore, pp. 431–441. https://doi.org/10.1007/978-981-15-1636-8_25.