

Laboratory based efficacy of Citrus limon mediated Ag-Pd bimetallic nanoparticles against mosquito larvae

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Research Article

Keywords: Bimetallic nanoparticles, biological synthesis, mosquito control, nanotoxicity, larvicidal efficacy

Posted Date: August 28th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4808828/v1>

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Bioscience Nanotechnology on February 7th, 2025. See the published version at <https://doi.org/10.1186/s44331-025-00001-7>.

Abstract

The current study presents data on the eco-friendly biological synthesis of silver and palladium bimetallic nanoparticles (Ag-Pd BNPs) using a 10% aqueous leaf extract of plant *Citrus limon*. The synthesized Ag-Pd BNPs were characterized using various techniques, including UV-Visible (UV-Vis) and Fourier transform infrared (FT-IR) spectroscopy, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy (SEM-EDX), Dynamic light scattering (DLS), and X-ray diffraction (XRD). Subsequently, the larvicidal nanotoxicity of the characterized Ag-Pd BNPs was assessed against I, II, III, and IV instar larvae of *Anopheles stephensi* and *Aedes aegypti* mosquitoes, and its impact on the predation efficiency of non-target nymphs of dragonfly and damselfly was evaluated. The results of UV-Vis displayed a maximum absorption at $300 \text{ nm} \pm 40 \text{ nm}$ for the leaf extract, while for the synthesized Ag-Pd BNPs no surface plasmon resonance band was observed. FTIR analysis revealed the involvement of surface functional groups from the leaf extract in nanoparticle synthesis. TEM analysis determined a mean particle size of $21 \pm 7.22 \text{ d nm}$, while DLS analysis confirmed a Z-average of 1956 d nm . SEM-EDX verified the presence and purity of the Ag-Pd BNPs in the sample, and XRD analysis identified the leaf extract-mediated synthesis with distinct peaks obtained for Ag and Pd. The nanotoxicity bioassay revealed significant larval mortality and the LC_{50} values were obtained at the time intervals of 24 h, 48 h, and 72 h of exposure for I to IV instars of both mosquito species. The predation efficiency test against the non-target nymphs of dragonfly and damselfly revealed time-dependent predation dynamics, resulting in high predation rates over specific time intervals. In conclusion, this study offers significant scientific insights into the characterization and ecotoxicological properties of Ag-Pd BNPs, demonstrating their potential under vector control while considering their impact on non-target organisms.

Introduction

The recent studies have demonstrated significant advancements in the field of nanotechnology, particularly in the domain of utilizing eco-friendly, cost-effective, and biological synthesis methods for the production of metal nanoparticles (NPs) [1]. These metallic NPs can be composed of a single metal or a bimetallic combination [2]. The unique physicochemical properties of NPs have significantly augmented their utilization in diverse applications. The metal NPs are being widely studied in the areas like efficient energy harvesting [3], photocatalytic effect [4], strength enhancing anti-corrosive surface coatings [5, 6], development of new generation printing electronics [7], nanosensors [8, 9], toxicity evaluation [10], and is not limited to its biomedical photo-thermal theranostics and imaging applications [11–13]. Among other metal nanoparticles, silver (Ag) NPs and gold (Au) NPs have been extensively studied through biological synthesis methods and have demonstrated toxic efficacies with a wide range of properties like antimicrobial [14, 15, 16], anti-fungal [17, 18], anti-larval [19, 20], and pesticidal activities[21], along with catalytic, imaging, therapeutic, and anticancer activities etc. [22, 23]. The Ag NPs synthesized from different leaf extracts exhibit diversity in terms of size and shape, contingent upon the specific constituents present in the leaf extract used to reduce the metal precursor agents into metal

NPs [24, 25]. The toxic efficacy of these Ag NPs may also vary due to the intrinsic toxic properties of the leaf extract used for their synthesis. Therefore, the utilization of a non-toxic reducing agent can facilitate a more accurate and effective evaluation of the toxicity of the selected metal NPs sample [26]. The utilization of leaf extract-mediated palladium (Pd) NPs has garnered significant attention following studies on Ag NPs and Au NPs, primarily due to their distinct physicochemical properties and size-dependent catalytic behaviour [27]. Studies have revealed that Pd may often exhibit synergistic effects on the overall properties of the biologically synthesized bimetallic nanoparticles (BNPs) when combined with growing seedlings of other metals, such as silver and gold. This imparts the BNPs a distinct morphology and catalytic activity compared to their monometallic counterparts [28, 29, 30]. The biological synthesis of Ag-Pd BNPs has been investigated using various plant tissue extracts, such as the leaf extracts of, *Catharanthus roseus* demonstrated photocatalytic dye degradation activity against safranin O textile dye, *Cacumen platyclade* exhibited catalytic hydrogenation of 1,3-butadiene, while the leaf extract of *Lithodora hispidula* showed electrocatalytic reduction of hydrogen peroxide, and the gum kondagogu extract of *Cochlospermum gossypium* displayed catalytic potential in the degradation of 4-nitrophenol [31, 32, 33, 34]. Moreover, the nut extract of *Prunus amygdalus*, as well as the fruit extracts of blackberry and *Terminalia chebula*, exhibited anticancer and antimicrobial activities [35, 36]. Lastly, Ag-Pd BNPs synthesized using a purified analytical grade biological pigment, rutin, has been successfully evaluated for its catalytic activity for the etherification reaction. The combined effects of Ag-Pd BNPs showed distinct properties compared to their monometallic forms, underscoring the need for further exploration of their activity in novel domains [37]. Considering the growing interest in the potential applications of Ag-Pd BNPs with antimicrobial activity, it is crucial to assess their ecotoxicity and potential use as an alternative to insecticides which is also a prerequisite to their environmental applications. One of the most thoroughly investigated applications of nanoparticles lies in evaluating their larvicidal properties against ectoparasitic mosquitoes as the target organisms [38–44]. These mosquitoes, particularly adult females of the *Anopheles* species, play a pivotal role in transmitting various strains of the Plasmodium parasite, responsible for causing different types of malaria in humans and animals. In 2021, malaria accounted for 247 million positive cases and 619,000 deaths worldwide [45]. During the same period, there were documented cases and fatalities of diseases such as chikungunya, dengue, rift valley fever, yellow fever, and Zika virus fever, all transmitted by *Aedes* mosquitoes species, which act as vectors for these pathogens. [46, 47]. A problem of climate change has led to the emergence of new breeding sites for adult mosquitoes in regions previously considered uninhabitable, as well as temporal shifts in breeding seasonality, has significantly influenced the dynamics of vector populations. Consequently, this raises substantial concerns regarding the potential burden of the emergence of vector-borne diseases in the future [48, 49, 50, 51]. With the emergence of insecticide-resistant population of mosquito species and frequent occurrence of mosquito-borne diseases, it is crucial to search for alternative insecticidal tools for vector control, in addition to other integrated management approaches [52]. Moreover, ecotoxicological studies are now focused on analyzing the effects of NPs against the non-target groups, including the natural enemies of mosquito such as nymphs of water bugs and odonates, crustaceans, and mosquito larvivorous fishes. This evolving field of study seeks to determine the minimal optimal concentration that ensures effectiveness

without detriment to the natural biocontrol agents of mosquito vectors [53, 54, 55]. In this study, we successfully synthesized and characterized Ag-Pd BNPs using a 10% aqueous leaf extract of the plant *Citrus limon*, known for its eco-friendly and non-toxic properties. We assessed the toxic efficacy of the synthesized Ag-Pd BNPs against *Anopheles stephensi* and *Aedes aegypti* mosquito larvae. The larvicidal potential was examined across various instar larvae (I, II, III, and IV), and the lethal concentration (LC₅₀) was calculated for each mosquito species. Additionally, we investigated the impact of the obtained LC₅₀ values on the predation rates of native dragonfly and damselfly nymphs to determine any potential non-target activity of the synthesized Ag-Pd BNPs.

Methods

All the methods were carried out in accordance with the Institutional guidelines and regulations.

Material. The study utilized analytical grade Silver nitrate (Merck Millipore), Palladium (II) chloride (HiMedia), hydrochloric acid (Merck), Whatman filter paper no.-1, and triple deionized (DI) water. For the larval bioassay, Temper (EC) (Temephos) insecticide was employed as the positive control. The biological specimens, including fresh leaves of *Citrus limon* (Linnaeus) Burm. f., mosquito larvae of *Anopheles stephensi* (Liston) and *Aedes aegypti* (Linnaeus), as well as nymphs of Dragonfly (*Bradinopyga geminata*) and Damselfly (*Ischnura senegalensis*), were collected from the campus of Dayalbagh Educational Institute (DEI) in Agra, India (27°, 10' N, 78° 05' E).

Preparation of phytochemical extract. The bioactive constituents present in the leaves of the plant *Citrus limon*, were extracted using the hot-pot method [30]. In this method, 10 gm of minced leaves of plant *Citrus limon* were added to an Erlenmeyer flask containing 90 ml of DI water and heated at 65–70°C for 1 h on a hot-plate. Subsequently, the plant debris was separated from the mixture, and the resulting crude extract was filtered using Whatman filter paper no.-1 to obtain a purified 10% aqueous leaf extract, used for nanoparticles synthesis, stored at 4°C.

Synthesis of Ag-Pd BNPs. To synthesize Ag-Pd BNPs, 1 mM solutions of Silver nitrate (AgNO₃) and Palladium (II) chloride (PdCl₂ – HCl dissolved) were prepared in DI water and were separately mixed with the prepared 10% aqueous leaf extract of *Citrus limon*, in a ratio of 9:1. The mixtures were then kept in a dark environment for 1 h. Subsequently, the active solution of pre-Ag NPs was combined with the active solution of pre-Pd NPs in a 1:1 ratio. The resulting solution was a 2 mM pre-Ag-Pd BNPs solution, which was kept in the dark at room temperature overnight and then stored at 4°C.

Characterization. The prepared Ag-Pd BNPs (2 mM) and aqueous leaf extract (10%) samples were diluted in DI water in 1:10 and 1:9 ratios, respectively, and were characterized after 48 h of synthesis. Thereafter, the optical spectroscopy analysis with UV-Visible (UV VIS) spectrophotometer (Hitachi U-3900) and Fourier transform infrared (FT-IR) spectrophotometer (Bruker TENSOR 37 FTIR) for both leaf extract and Ag-Pd BNPs was performed. The micrographs of Ag-Pd BNPs (2 mM) were generated using Transmission Electron Microscopy (TEM) (Technai G 20 (FEI) TEM), and the sample was analyzed over

formvar coated copper grids. The Ag-Pd BNPs sample was air-dried on the carbon tape substrate in a desiccator and was analyzed by Scanning Electron Microscope (SEM), the micrographs were obtained and the visualized area was further studied to determine the presence and composition of the Ag-Pd BNPs sample using Energy Dispersive X-ray (EDX) spectroscopy (SEM – Zeiss EV040 with PANalytical X'pert PRO). The hydrodynamic size of Ag-Pd BNPs was measured using Dynamic Light Scattering (DLS) analyzer (Malvern Zetasizer Ver. 7.12), and the crystalline nature of the nanoparticles was evaluated using X-ray Diffractometer (XRD) (D8 ADVANCE Bruker).

Preparation of mosquito larvae for bioassay. The collected larvae were initially separated into batches according to their developmental stages: II, III, and IV instar. To ensure accurate identification, online identification keys provided by the Walter Reed Biosystematics Unit (WRBU) were utilized [56]. The source water was further kept in a bio-incubator at 28°C with a photoperiod of 8:16 h (L/D), to facilitate the hatching of newly emerged I instar larvae for both mosquito species. Prior to the bioassay, the mosquito larvae were washed twice with distilled water and morbid larvae were identified and removed from the batches to maintain a healthy and uniform larval population for the experiments.

Nanotoxicity Bioassay. The evaluation of Ag-Pd BNPs in terms of nanotoxic efficacy was conducted following the guidelines outlined in the World Health Organization's manual on testing of mosquito larvicide (2005) [57]. The bioassay was performed against I to IV instar larvae of both *Anopheles stephensi* and *Aedes aegypti* mosquito species. A wide range of test concentrations containing 25 larvae were assessed, and a specific range of concentrations was selected based on achieving 50% mortality after 24 h. The selected concentrations were 1.67, 3.34, 5, 6.67, and 13.34 mL/L of Ag-Pd BNPs were prepared in 150 mL of distilled water in a glass beaker. For each concentration, triplicates were prepared, each containing 25 larvae of same instars (either I, II, III, and IV) for both mosquito species, separately. For control groups, a positive control comprising 0.2% Temephos (EC) was used, along with two negative controls. One negative control consisted of 150 mL of distilled water, while the other negative control contained 13.34 mL/L of the prepared aqueous leaf extract (10%). The mortality rates within each test concentration and control groups were assessed at 24 h, 48 h, and 72 h or until 100% mortality was observed. The resulting data was analyzed to determine the effective lethal concentration (LC₅₀), which represents the concentration at which 50% mortality occurred in the test concentration.

Preparation of predatory nymphs. The collected nymphs of Dragonfly (*Bradinopyga geminata* (Rambur, 1842)) [58] and Damselfly (*Ischnura senegalensis* (Rambur, 1842)) [59] were identified using pictorial keys available in the Indian Biodiversity Portal. The identification process ensured accurate classification of the nymphs. Following identification, the nymphs of both species were individually placed in separate vessels. The vessels were provided with mosquito larvae, serving as a food source for the nymphs. This arrangement allowed the nymphs to acclimate and adapt to their environment, ensuring their readiness for the subsequent predator efficiency test.

Predation efficiency test. The rate of consumption of mosquito larvae by the predatory nymphs in the Ag-Pd BNPs test groups compared to the negative control groups was recorded to evaluate the predation

efficiency. The nymphs of both dragonfly and damselfly measuring up to 1 inch in length, were selected as non-target test subjects. The LC_{50} value obtained from the larvicidal nanotoxicity bioassay against III instar larvae of both *Anopheles stephensi* and *Aedes aegypti*, at 24 h of exposure were selected for Ag-Pd BNPs test. Subsequently, four combinations (*Anopheles stephensi* with Dragonfly nymph, *Anopheles stephensi* with Damselfly nymph, *Aedes aegypti* with Dragonfly nymph, and *Aedes aegypti* with Damselfly nymph) were made by adding 25 III instar larvae and a nymph in a 250 mL glass beaker containing 150 mL of distilled water. The negative control contained only mosquito larvae and a nymphs while the Ag-Pd BNPs test was obtained by adding the respective LC_{50} values to the prepared combinations. Each test and negative control (distilled water) were prepared in replicates (R1, R2, R3). The total predation process was observed at specific time intervals of 16 h, 20 h, 24 h, 40 h, 48 h, 64 h, and 72 h. To determine the % of predation efficiency achieved in each test and control groups, the formula: $((\text{Number of consumed mosquito larvae} / \text{Number of predators}) / \text{Total number of mosquito larvae}) \times 100$, was employed. [60, 61].

Data analysis. The micropictographs obtained from TEM analysis were further analyzed using ImageJ software to generate a size distribution histogram of the nanoparticles, for the calculation of the mean particle size (in diameter nm) $\pm$ S.D. (standard deviation) [62]. The raw mortality data obtained from the nanotoxicity bioassay of Ag-Pd BNPs was subjected to Abbott's method (1925) [63] to determine the corrected mean mortality (%), i.e., when 5–20% mortality was observed in the negative control. This corrected mean mortality (%) values were then transformed into their respective probit values using the probit table (Finney 1948) [64]. Probit Analysis (Finney in 1952) [65] was used, the regression graphs were plotted for obtained probit values and the probit equations ($\hat{y}$ -value) were generated to obtain LC_{50} values with its lower confidence limit (LCL) and upper confidence limit (UCL) at 95% Fiducial Confidence Interval (CI), through regression analysis using MS EXCEL. Additionally, $LC_{50} \pm$ S.D. (standard deviation) and S.E. (standard error), chi-squared (χ^2) test for the goodness of fit, and two-way ANOVA was also calculated. The transmittance peaks of FT-IR spectroscopy were analyzed as per the standard keys provided by Coates (2000) [66]. The graphs for characterization and probit analysis were plotted using the OriginPro 2023b [67].

Results and Discussion

UV VIS spectroscopy analysis. The absorption spectra of the diluted samples (1:9) of aqueous leaf extract (10%) of plant *Citrus limon* (pH 5.29 at 29°C) exhibited the maximum absorbance in the UV region around 300 nm $\pm$ 40 nm (Fig. 1.A.a). Whereas, the diluted samples (1:10) of Ag-Pd BNPs (pH 1.26 at 29°C) showed absorption in the range of 380–500 nm without any distinct surface plasmon resonance (SPR) band (Fig. 1.A.b). Generally, biologically synthesized Ag NPs exhibit a characteristic SPR band around 390–450 nm [19], while Pd NPs solution shows an absence of SPR band but presence of absorption in the visible region, unlike the molar solution and leaf extract [43]. The absence of SPR band in the current results aligns with the results of Ag-Pd BNPs synthesized using *Catharanthus roseus* leaf extract having an equal (1:1) Ag to Pd ratio by Mohan et al. (2021) [31]. This phenomenon of the

absence of SPR band can be attributed to the combined effect of bimetallic properties resulting from the formation of Ag-Pd BNPs, where the presence of Pd acts as a shield, this was attributed to the exceptional electronic configuration of Pd, which influences the SPR properties of Ag within the BNPs, also observed for the Ag-Pd BNPs synthesized using fruit extract of *Terminalia chebula* by Sivamaruthi et al. (2019) [36]. Compared to our studies, Abdel-Fattah et al. (2017) [35], observed that the absence of the SPR band is linked with the Ag to Pd ratio. The use of relatively lower Ag concentrations, Pd:Ag (1:2), shows absence of SPR band, consistent with our findings, which involved an equal concentration of both Ag and Pd (1:1). They further reported a prominent SPR band appeared in the formulation with higher Ag concentration (1:3, 1:4, 1:6 and 1:8), for the almond nut and blackberry fruit extract mediated Ag-Pd BNPs samples.

FT-IR spectroscopy analysis. Leaf Extract: The infrared spectrum of the aqueous leaf extract (Fig. 1.B.a) revealed a transmittance band with a broad peak (3700 cm^{-1} - $\sim 2800\text{ cm}^{-1}$) corresponding to the stretching vibrations of the functional groups. These include O-H groups of intermolecular (high intensity peak) and intramolecular (weak intensity peak) bonded alcohols ($3550 - 3200\text{ cm}^{-1}$), N-H groups of amine salts ($3000 - 2800\text{ cm}^{-1}$), and overlapping C-H stretching vibrations of the alkane ($3000 - 2840\text{ cm}^{-1}$), alkene ($3100 - 3000\text{ cm}^{-1}$), and alkyne ($3333 - 3267\text{ cm}^{-1}$). A weak intensity peak around $2140 - 2100\text{ cm}^{-1}$ shows $\text{C} \equiv \text{C}$ stretching of monosubstituted alkyne compounds, and C-H bending of aromatic compounds is present around $2000 - 1650\text{ cm}^{-1}$. A medium-intensity peak showing $\text{C} = \text{C}$ stretching of conjugated alkene ($1650 - 1600\text{ cm}^{-1}$), alkene di-substituted (cis) ($1662 - 1626\text{ cm}^{-1}$), cyclic alkene ($1650 - 1566\text{ cm}^{-1}$), and N-H bending was observed for amines ($1650 - 1580\text{ cm}^{-1}$). Another medium intensity peak for O-H bending of phenol compounds ($1390 - 1310\text{ cm}^{-1}$) is also observed and a characteristic strong intensity peak in the fingerprint region ($1000 - 650\text{ cm}^{-1}$) indicating the stretching and bending vibrations for C-H bending ($900 - 700\text{ cm}^{-1}$), $\text{C} = \text{C}$ bending of alkene vinylidene compounds ($895 - 885\text{ cm}^{-1}$), and stretching vibrations of the halo compound groups such as C-Cl, C-Br, and C-I can be seen around $850 - 550\text{ cm}^{-1}$, along with a peak for complex benzene derivatives around $700\text{ cm}^{-1} \pm 20\text{ cm}^{-1}$. **Ag-Pd BNPs:** In contrast, the FT-IR spectra of Ag-Pd BNPs (Fig. 1.B.b) exhibited a highly reduced transmittance band between 3700 cm^{-1} - $\sim 2800\text{ cm}^{-1}$ compared to the leaf extract sample. The $\text{O} = \text{C} = \text{O}$ stretching (2349 cm^{-1}) of carbon dioxide was observed, and a comparatively reduced fingerprint region ($1000\text{ cm}^{-1} - 599\text{ cm}^{-1}$) with weak intensity peaks can be seen in contrast to the strong intensity band of leaf extract. This change can be attributed to the utilization of the specific surface functional groups of leaf extract during nanoparticles synthesis. These results are consistent with the reduced transmittance band of infrared spectra observed for the samples of *Cochlospermum XXXossypium* gum kondagogu extract mediated Ag-Pd BNPs (Velpula et al. (2021)) [34].

TEM analysis. The micrographs of Ag-Pd BNPs samples (Fig. 2.A) revealed the presence of polydispersed electron-dense metal nanoparticles. These nanoparticles exhibited roughly irregular-spherical shape and displayed minimal agglomeration. The size distribution bins of the histogram

(Fig. 2.B) generated for the TEM micrograph showed particles sizes ranging from 5–34 d nm (diameter in nm). The Gaussian fit analysis for the obtained particle size distribution bins showed a mean particle size ($\pm$ SD) of 21 ± 7.22 d nm. The morphological variation in the size of biologically synthesized Ag-Pd BNPs using different extracts and methods is evident in the literature. Factors such as the type of extract used, Ag to Pd ratio, and duration of synthesis seem to play a role in determining the characteristic features of the synthesized BNPs and their properties for functions such as catalysis, antimicrobial activity, and anticancer effects, among others. The observed particles size in our study aligns with the findings of Sivamaruthi et al. (2019) [36], who successfully synthesized Ag-Pd BNPs (2–40 nm) with an average particles size of ~ 20 nm, using 1% *Terminalia chebula* fruit extract by reducing 10 mM AgNO₃ for 1 h, followed by the addition of 10 mM PdCl₂ in a 1:1 ratio. Similarly, Velpula et al. (2021) [34] reported comparable results using 1% gum kondagogu extract mediated Ag-Pd BNPs (2–40 nm) with a mean particles size of ~ 21 nm, obtained by reducing combined precursor solution of 1mM AgNO₃ and PdCl₂ in 1:1 ratio. In another study, Mohan et al. (2021) [31] demonstrated the synthesis of Ag-Pd BNPs (1 mM, 1:1) using a 1% methanolic leaf extract of *Catharanthus roseus*, the resultant NPs exhibited a size distribution spanning 23–64 nm, with an average particles size falling within the range of 15–30 nm. In 2014, Lu et al. [32] reported the synthesis of monometallic nanoparticles mediated by *Cacumen platyclade* leaf extract. These nanoparticles exhibited an average particle size of 22 nm for Ag NPs and 7.3 nm for Pd BNPs. Additionally, the synthesis of bimetallic nanoparticles with various Ag to Pd ratios yielded particle sizes of 11.9 nm (3:1), 9.1 nm (1:1), and 7.4 nm (1:3) for Ag-Pd BNPs. Their findings indicated that a higher Pd concentration resulted in smaller particle sizes, which, in turn, demonstrated enhanced catalytic activity when compared to other nanoparticles.

DLS analysis. The Zetasizer results (Fig. 3A) for the diluted samples (1:10) of the Ag-Pd BNPs (2 mM) revealed a hydrodynamic size range of 800–1050 d nm. The Z-average was measured at 1956 d nm, with the peak ($\pm$ S.D.) observed at 982.2 d nm (± 142.8 d nm), and a polydispersity index (PDI) of 0.515. This suggests that the sample has a larger hydrodynamic size when compared to the actual size of the metal NPs obtained from TEM analysis. In comparison to our results, a smaller hydrodynamic size of the biologically synthesized Ag-Pd BNPs was reported by Mohan et al. (2021) [31] in the range of 2–28 d nm with an average size of 28 d nm, while Velpula et al. (2021) [34] determined an average particles size of 44.8 d nm, and Sivamaruthi et al. (2019) [36] reported a range of 5-200 d nm with Z-average of 31.93 d nm. Turunc et al. (2017) [33] reported the average particle size of 15 d nm for Ag NPs and 22 d nm for Pd NPs, indicating larger hydrodynamic size of smaller Pd NPs solution compare to Ag NPs. The DLS chart obtained by Abdel-Fattah et al. (2017) [35], showed the presence of Ag-Pd BNPs in the size range of 90-1200 d nm, which is close to our results. Yeap et al. (2018) [68] suggest that samples containing active interacting molecules tend to aggregate, resulting in larger-sized particles during DLS analysis compared to TEM analysis. According to Souza et al. (2016) [69] and Bhattacharjee (2016) [70], the aggregating properties of leaf extract biomolecules, which are used as capping and reducing agents, often contribute to NP retention and control their release in the environment.

XRD analysis. The results of XRD pattern for Ag-Pd BNPs samples (Fig. 3.B) revealed distinct peaks at 38.7°, 44.6°, 64.7°, and 77.4° corresponding to 2θ angle with lattice constants of (111), (200), (220) and (311), respectively. These peaks signify the presence of Ag particles in the sample, in accordance with the Ag content indicated by JCPDS no. 87–0720. Additionally, peaks at 40.5°, 48.6° and 68.4° of 2θ angle with lattice constants of (111), (200), and (220), respectively, were observed, indicating the presence of palladium in the sample as confirmed by JCPDS no. 65-6174. The high background noise and low-intensity peaks observed indicate the semi-crystalline nature of the Ag-Pd BNPs sample. This can be attributed to the synthesis process using leaf extract and low-concentration Ag-Pd BNPs present in the test sample. Similar to our results, the Ag-Pd BNPs synthesized using the leaf extract of *Cacumen platycladi* (Lu et al., (2014)) [32], gum kondagogu extract of *Cochlospermum gossypium* (Velpula et al. (2021)) [34], and rutin biomolecule mediated synthesis showed identical X-ray diffractogram (Singh et al. (2020)) [37].

SEM-EDX analysis. The illuminating surface containing Ag-Pd BNPs samples of SEM micrograph (Fig. 4.b) confirms the presence of metal nanoparticles. The peaks corresponding to Ag and Pd elements are present with an absence of the peaks related to other metal contaminants. The energy table (Fig. 4.a) generated from the EDX analysis of SEM micrograph shows the data generated from the L-series confirms the higher element % for silver (Ag) (88.74) as compared to palladium (Pd) (11.26), and with the atomic % of 88.60 for Ag and 11.40 for Pd, in the composites of Ag-Pd BNPs samples. This shows the content and composition of the formed nanoparticles (Ag-Pd BNPs), having a larger content of Ag as compared to Pd in the sample of formed Ag-Pd BNPs.

Nanotoxicity Bioassay. The results of the Ag-Pd BNPs bioassay showed toxic efficacies against the I, II, III, and IV instar larvae of selected mosquito species. The negative control containing distilled water and leaf extract showed no morbidity or mortality till 72 h of exposure. The positive control showed 100% mortality after 4 h. The mean corrected % mortality obtained [refer to Table S1 in supplementary material] from the bioassays was subjected to probit analysis and probit regression plots were generated for both *Anopheles stephensi* (Fig. 5.A) and *Aedes aegypti* (Fig. 5.B) for the calculation of the LC₅₀ (LCL-UCL) values with ± S.D. and S.E., against both mosquito species (Fig. 6.A.B) at 24 h, 48 h, and 72 h of exposure [refer to Table S2 and S3 in supplementary material]. For the Ag-Pd BNPs bioassay against *Anopheles stephensi*, the non-significant (NS) value of χ^2 test depicting the goodness of fit were obtained for the test concentrations against II, III, and IV instar larvae after 24 h of exposure, for II instar larvae LC₅₀ (LCL-UCL) of 1.59(1.08–2.33) mL/L was observed with ± 0.33 S.D and 0.09 S.E., for III instar larvae LC₅₀ of 1.78(1.21–2.60) mL/L was observed with ± 0.36 S.D. and 0.08 S.E., and for IV instar larvae LC₅₀ of 1.62(1.12–2.34) mL/L with ± 0.31 S.D. and 0.08 S.E. was observed. While 100% mortality was observed for I instar larvae at 24 h in the lowest selected concentration and significant χ^2 values (bad-fit) were obtained for the efficacy data against I instar larvae at 24 h, and for II, III, and IV instar larvae at 48 h and 72 h of exposure. The results obtained for Ag-Pd BNPs bioassay against *Aedes aegypti* showed the non-significant (NS) χ^2 test values depicting goodness of fit for the LC₅₀ generated from the test concentrations against II (24 h), III, and IV instar larvae (24 h and 48 h), for II instar larvae LC₅₀ of

1.59(1.08–2.33) mL/L was observed with ± 0.33 S.D and 0.09 S.E. (24 h), for III instar larvae LC_{50} of 1.78(1.21–2.60) mL/L with ± 0.36 S.D. and 0.08 S.E. at 24 h and LC_{50} of 0.51(0.23–1.13) mL/L with ± 0.72 S.D. and 0.18 S.E. at 48 h was observed, and for IV instar larvae, the LC_{50} of 2.08(1.37–3.16) mL/L with ± 0.44 S.D. and 0.09 S.E. at 24 h and LC_{50} of 1.36(0.87–2.14) mL/L with ± 0.40 S.D. and 0.10 S.E. at 48 h was observed. The results of two-way ANOVA showed a significant difference between the dependent variable i.e., obtained LC_{50} values after 24 h of exposure, against the independent variable i.e., mosquito species and larval Instar (II, III, and IV) [refer to Table S4 and S5 in supplementary material]. Presently, the absence of comparative data for mosquito larvicidal bioassays involving Ag-Pd BNPs makes it challenging to establish a comparative relationship between particles size, toxicity (LC_{50}), and the type of extract used. However, our findings lay the groundwork for future comparisons of the efficacies of biologically synthesized Ag-Pd BNPs against larvae of mosquito vector. Erstwhile, the monometallic NPs of Ag and Pd are well known to show toxic efficacies against mosquito larvae. One of the most effective formulations, containing an optimum concentration of Ag NPs, synthesized from the entomopathogenic fungus *Chrysosporium tropicum*, demonstrated larvicidal activity against *Culex quinquefasciatus* and *Anopheles stephensi*. The entomopathogenic nature of the fungus is advantageous for the biological management of insect pests. Furthermore, the addition of an optimal concentration of Ag NPs to the fungal broth has been shown to synergistically enhance the toxicity of the formulation, rather than inhibiting fungal growth. This enhancement extends the shelf life of the formulation thereby promoting its adhesion to the insect cuticle, leading to fungal infestation in the insect pest. Such strategic formulations contribute to reducing environmental implications by decreasing reliance on chemical insecticides and enhancing the effectiveness of biological control methods (Soni and Prakash (2012)) [71]. Bhakyara et al. (2017) [72], observed that the Pd NPs synthesized using a toxic leaf extract of plant *Melia azedarach*, showed greater larvicidal toxicity against III instars of *Aedes aegypti* with LC_{50} obtained at 27.36% as compared to the leaf extract alone with LC_{50} at 93.96% at 24 h. Therefore, the combined toxic effect with the leaf extract can contribute to the overall toxicity which can be further enhanced by the additional synthesized Pd NPs in an optimum concentration. Conversely, Minal and Prakash (2018) [43], used a non-toxic leaf extract of *Citrus limon* for Pd NPs synthesis and reported an LC_{50} value of 16.038% at 24 h of exposure against III instar larvae of *Anopheles stephensi*, thus emphasizing the intrinsic toxic efficacy of Pd NPs without a significant contribution from the leaf extract. An attempt to elucidate the mechanism of action leading to larval mortality induced by these nanoparticles was conducted by Kalimuthu et al. (2017) [73] through histological studies of *Hedychium coronarium* rhizome-mediated Ag NPs treated IV instar larvae of *Aedes aegypti*. Their findings indicated that, in comparison to the untreated control group, the mid-gut of the treated larvae exhibited partial lysis of the epithelium at the apical side. This was attributed to swollen epithelial cells filled with multiple vacuoles, and the lumen contained the content of ruptured epithelial cells. The physiological damages caused by Ag NPs were further analyzed at the cellular and biochemical levels by Fouad et al. (2017) [74], using *Cassia fistula* fruit pulp extract-mediated Ag NPs against the IV instar larvae of *Aedes albopictus* and *Culex pipiens pallens*. They reported a considerable decrease in the total protein content and downregulation of two key enzymes, namely Acetylcholinesterase and α - and β -carboxylesterase. In

addition to these three biochemical parameters, Ga'al et al. (2017) [75] also reported the downregulation of acid and alkaline phosphatase due to Ag NPs synthesized with the green method using salicylic acid and its derivative, 3,5-dinitrosalicylic acid. These changes demonstrate the direct toxic effect of the Ag NPs, resulting in abnormal physiology, cell signalling alterations, often leading to cell lysis, and a reduction in metabolic and neuromuscular activity. Moreover, the inability of downregulated and damaged α - and β -carboxylesterase enzymes to further detoxify the cell leads to the accumulation and aggregation of the metal NPs in the system, which is the main cause of larval mortality in the nanotoxicity bioassay. Thereby, making NPs the most suitable alternative against mosquito species resistant to chemical and biological insecticides, while simultaneously raising concerns about its effects on other non-target environmental organisms.

Predation efficiency test. The number of larvae consumed by each selected predatory nymphs were recorded for Ag-Pd BNPs test (LC_{50}) and negative control in replicates (R1, R2, R3) containing 25 III instar larvae of both mosquito species [refer to Table S6 and S7 in the supplementary material]. The negative control groups, comprising of a dragonfly nymph with 25 III instar larvae of *Anopheles stephensi* exhibited 100% predation within 24 h (Fig. 7.A.a) and for *Aedes aegypti*, a complete predation was observed at 40 h (Fig. 7.A.b). Conversely, the negative control groups consisting of a damselfly nymph with 25 III instar larvae of *Anopheles stephensi* in each replicate, demonstrated 100% predation at 64 h (Fig. 7.A.c), whereas for *Aedes aegypti*, complete predation occurred at 40 h (Fig. 7.A.d). The LC_{50} value of 1.78 mL/L, obtained from the nanotoxicity bioassay using III instar larvae, was specifically selected as the benchmark for assessing the predation efficiency of the non-target organisms. For Ag-Pd BNPs (LC_{50}) test containing a Dragonfly nymph and 25 III instar of *Anopheles stephensi*, the predation of 55% larvae at 16 h was observed, which increased to 71% at 20 h, 92% at 24 h, and ultimately reached 100% at 40 h (Fig. 7.B.a) and for *Aedes aegypti*, 61% larval predation at 16 h, rising to 73% at 20 h, 95% at 24 h, and achieving complete predation (100%) at 40 h (Fig. 7.B.b). The Ag-Pd BNPs (LC_{50}) test against the nymph of Damselfly with 25 III instar of *Anopheles stephensi*, showed consumption of 51% of larvae at 16 h, which increased to 84% at 20 h, subsequently to 92% at 24 h, and ultimately achieved complete predation (100%) at 40 h (Fig. 7.B.c), and for *Aedes aegypti*, the 76% larval predation at 16 h, increased to 93% at 20 h, and eventually complete predation (100%) at 24 h was observed (Fig. 7.B.d). The results obtained from the predation tests demonstrate a significant improvement in predation efficiency for the Ag-Pd BNPs test compared to the untreated control groups. The predatory nymphs exhibited resilience to the applied test concentration, as no mortality was recorded in any of the replicates during the 72 h observation period. The increased predation rates, however, may be a response to meet the energy demand for defence against the metal stress induced by the Ag-Pd BNPs test in the nymphs. dos Santos Lima et al. (2021) [76] investigated disruptions in the food web caused by metal stress from copper, cadmium, mercury, and manganese. The study aimed to examine how this metal stress influenced biotic interactions, specifically focusing on the feeding preferences and predation rates of dragonfly nymphs (*Tamea cophisa*) and ostracods (*Chlamydotheca* sp.) on water-dwelling crustaceans. The research revealed that, although metal stress did not alter food preferences, it did modify predation behaviour in a species-specific and metal-specific manner. Overall, a decrease in predation rates was observed for

dragonfly nymphs, while increased predation rates were noted for ostracods. Hence, this study establishes that the enhanced predation rate of nymphs induced by nanoparticles may not necessarily be beneficial for the well-being of the predatory nymph. Azam et al. (2015) [77] found the libellulid dragonfly (*Crocothemis servilia*) to be a highly effective ecological indicator for water and riparian systems. They observed that heavy metal-induced stress not only alters the life history of insects but also makes them efficient bio-indicators due to metal bioaccumulation, offering a clear measure of heavy metal contamination across different locations. Similarly, a study conducted by Akhtar et al. (2021) [78] demonstrated the occurrence of bio-transfer and bioaccumulation of various heavy metals between the trophic levels of the food chain from mosquito larvae to natural predators, such as dragonfly nymphs (*Tamea cophysa*), frequently resulting in the mortality of nymphs due to higher metal bioaccumulation. A notable decrease in the mobility of mosquito larvae from both species under the applied nanotoxicity bioassay can be a factor influencing predation efficiency, contributing to the accelerated predation by nymphs within the experimental setup. Likewise, in a study conducted by Murugan et al. (2015a) [79], it was demonstrated that the specified lower concentration of synthesized Ag NPs could effectively induced mortality in *Anopheles* larvae, while it had no apparent impact on the predation efficiency of dragonfly nymphs (*Anax immaculifrons*). Studies conducted on natural copepod predators (*Mesocyclops longisetus*, *Mesocyclops aspericornis*, and *Megacyclops formosanus*) which are found to be effective against the I and II instar of *Anopheles* and *Aedes* mosquito species, revealed an increase in predation at lower test concentrations of Ag NPs without resulting in predator mortality. Conversely, higher concentrations of Ag NPs led to predator mortality, suggesting the need for lower doses to ensure that natural copepod predators are not adversely affected by Ag NPs (Murugan et al. (2015b) [80], Murugan et al. (2016) [81], Kalimuthu et al. (2017)) [82]. Therefore, the observed increase in predation rates in our study compared to the control group may be attributed to metal stress. While the selected concentration of Ag-Pd BNPs has not adversely affected the nymphs directly, their feeding on the Ag-Pd BNPs-affected, slowed larvae could lead to further bioaccumulation of nanoparticles from prey to predator through food chain. This process may eventually result in complications and the ultimate death of the natural biocontrol agents. Consequently, we recommend future studies on comparatively long-term investigation of the effects of nanoparticle bioaccumulation on the normal continuity of the life cycle non-target organisms.

Despite the absence of studies on biologically synthesized Ag-Pd BNPs against both target mosquito larvae and non-target predatory organisms, there are existing studies on the antimicrobial and anticancer activities of Ag-Pd BNPs. Abdel-Fattah et al. (2017) [35] synthesized Ag-Pd BNPs using almond nuts and blackberry fruit extracts separately, incorporating varying Pd (1) to Ag (2,4,6, and 8) ratios-specifically, 1:2, 1:3, 1:4, 1:6 and 1:8. The almond nuts extract-mediated Pd-Ag BNPs (1:6) and blackberry fruit extract-mediated Pd-Ag BNPs (1:8) were selected for TEM analysis, showing the formation of 8 nm and 6 nm nanoparticles, respectively. These Ag-Pd BNPs, mediated from both extracts, demonstrated cytotoxicity against human breast cancer (MCF7) and liver cancer (HEPG2). Notably, a Pd:Ag ratio of 1:3 exhibited maximum mortality at the half-maximal inhibitory concentration (IC₅₀), outperforming Ag-Pd BNPs with a Pd:Ag ratio of 1:8. This highlights the significant impact of the Pd to Ag ratios used in the

formulation. Additionally, in the antimicrobial assay against bacterial strains such as *Escherichia coli* and *Staphylococcus aureus*, as well as fungal strain of *Candida albicans*, Ag-Pd BNPs with a Pd:Ag ratio of 1:8 outperformed those with lower Ag ratios, shedding light on the cell-specific and composition-specific activity of Ag-Pd BNPs. Sivamaruthi et al. (2019) [36] observed that Ag-Pd BNPs mediated by *Terminalia chebula* fruit extract exhibited substantial antimicrobial activity against bacteria, including methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The increase in concentration of Ag-Pd BNPs from 20 µg/mL to 200 µg/mL showed a notable enhancement in the cytotoxic anticancer activity against the human lung cancer cell line (A549). Therefore, the antimicrobial properties of the Ag-Pd BNPs (~ 20 nm) derived from the non-toxic fruit extract of *Terminalia chebula* suggests that Ag-Pd BNPs (~ 21 nm) synthesized using the eco-friendly and non-toxic leaf extract of *Citrus limon* may also possess potential antimicrobial activity, attributed to their < 25 nm size. The catalytic potential of Ag-Pd BNPs appears promising and is subject to variation based on the differential composition in the Ag to Pd ratios. For instance, Lu et al. (2014) [32] reported the synthesis of Ag-Pd BNPs using *Cacumen platycladi* leaf extract in varying ratios of Ag:Pd, exhibited sizes of 11.9 ± 0.8 nm (3:1), 9.1 ± 0.7 nm (1:1), and 7.4 ± 0.4 nm (1:3) and demonstrated that Ag-Pd BNPs in a 1:3 ratio, supported by γ -Al₂O₃ catalyst, were particularly effective in the hydrogenation of 1,3-butadiene compared to the other two ratios of Ag:Pd. This study not only reveals the influence of different ratios on the mean particle size but also highlights the variations in catalytic efficiency among the formed formulations. A study on mono-and bi-metallic NPs of Ag and Pd was analyzed by Turunc et al. (2017) [33], they synthesized monometallic Ag NPs and Pd NPs, and bimetallic Ag-Pd BNPs using *Lithodora hispidula* leaf extract. These nanoparticles were employed to modify glassy carbon electrodes (GCE), resulting in the production of Ag NPs-GCE, Pd NPs-GCE, and Ag-Pd BNPs-GCE for the electrocatalytic reduction of hydrogen peroxide (H₂O₂). Among these, Ag-Pd BNPs-GCE demonstrated superior efficiency, attributed to its increased synergistic catalytic effect, and exhibited a low detection limit, making it a more promising material to produce an H₂O₂ nanosensor. The enhanced efficiency of Ag-Pd BNPs over Ag NPs and Pd NPs alone underscores the importance of bimetallic-based experimental studies to achieve optimum catalytic efficiency based on metal combinations. On the other hand, Singh et al. (2020) [37] demonstrated the synthesis of nanoparticles using a single biomolecule, rutin. This study also involved the synthesis of both monometallic Ag NPs (~ 50 nm), Pd NPs (~ 10 nm), and bimetallic Ag-Pd (1:1) BNPs (~ 80 nm). These nanoparticles exhibited distinct catalytic activities in etherification reactions with phenolic compounds, with the order of reactivity being Pd NPs > Ag NPs > Ag-Pd BNPs. Notably, the smaller particle size (Pd NPs) displayed the highest reactivity among the three types. This highlights the importance of size-specific catalytic activity of the nanoparticles. Achieving a lower size of Ag-Pd BNPs, as done by the extract of fruit extract of *Terminalia chebula* [36], gum kondagogu extract [34] and in the current study through leaf extract of *Citrus limon* mediated Ag-Pd BNPs, could enhance efficiency. Therefore, testing whether this enhanced activity is due to the properties of the metal or the size of the overall nanoparticles is also required. Mohan et al. (2021) [31] utilized *Catharanthus roseus* mediated Ag-Pd BNPs (15–30 nm), which exhibited a remarkable 98% photocatalytic degradation of safranin O textile dye within 40 minutes of exposure. Moreover, they demonstrated a free radical scavenging efficiency of 70.2% (Ag-Pd BNPs), surpassing the 48.2% achieved by the leaf extract. Here, the enhanced efficiency can be attributed to the

smaller size and unique properties of the synthesized Ag-Pd BNPs. Other combinations involving Au salts were compared by Velpula et al. (2021) [34], who demonstrated the synthesis of Ag-Au BNPs, Ag-Pd BNPs, and Au-Pd BNPs mediated by the gum kondagogu extract of the plant *Cochlospermum gossypium*. These nanoparticles were tested for their efficiency in catalyzing the reduction of 4-nitrophenol (4-NP). The results revealed catalytic efficiencies in the order of Ag-Pd BNPs (5–40 nm) > Ag-Au BNPs (2–40 nm) > Au-Pd BNPs (4–35 nm). This suggests that, for certain reactions, the properties dependent on the elemental composition are as significant as the size-related activity of the synthesized nanoparticles.

In contrast to our current findings on *Citrus limon*-mediated Ag-Pd BNPs, our previous studies on *Citrus limon*-mediated Au-Pd BNPs (Minal and Prakash (2020) [30]) exhibited unique characteristics and distinct bioassay results. UV-VIS spectroscopy analysis for Au-Pd BNPs revealed the presence of an SPR band around 540 nm due to the Au content, while the SPR band related to Ag was absent in the current Ag-Pd BNPs studies. The mean particle size of *Citrus limon*-mediated Au-Pd BNPs was $9.3 \text{ nm} \pm 3.95 \text{ nm}$, whereas our current studies observed a larger particle size of $21 \text{ nm} \pm 7.22 \text{ nm}$ for Ag-Pd BNPs. DLS analysis indicated a size range of 150–200 nm with a Z-average of 3340 nm for Au-Pd BNPs, compared to the present DLS analysis with a Z-average of 1956 nm and the hydrodynamic size range of 800–1050 nm for Ag-Pd BNPs. Despite utilizing the same reducing agent, the observed variation in particle size was evident. Furthermore, the efficacy of *Citrus limon*-mediated Au-Pd BNPs nanotoxicity bioassay against I, II, III, and IV instar larvae of *Anopheles stephensi* and *Aedes aegypti* showed higher LC_{50} values compared to the lower LC_{50} values observed in the present study of Ag-Pd BNPs nanotoxicity bioassay. The LC_{50} values against I – IV instar larvae of *Anopheles stephensi* in the previously reported Au-Pd BNPs test were 5.12 mL/L, 8.14 mL/L, 26.32 mL/L, and 11.4 mL/L at 24 h, respectively, while LC_{50} values of 0.1 mL/L, 1.59 mL/L, 1.78 mL/L, and 1.62 mL/L at 24 h, respectively, were observed for the current Ag-Pd BNPs test. Similarly, for the previously reported Au-Pd BNPs, LC_{50} values of 12.37 mL/L, 11.24 mL/L, 6.17 mL/L, and 10.83 mL/L at 24 h against *Aedes aegypti* were reported, compared to current Ag-Pd BNPs test with observed LC_{50} values of 0.1 mL/L, 1.59 mL/L, 1.78 mL/L, and 2.08 mL/L at 24 h, respectively. The predation efficiency of non-target nymphs of dragonfly and damselfly under the Au-Pd BNPs test (6.67 mL/L) showed a complete predation at 40 h for *Anopheles stephensi* with dragonfly nymph, while for other tests, including *Anopheles stephensi* with damselfly nymph, *Aedes aegypti* with dragonfly nymph, and *Aedes aegypti* with damselfly nymph, complete predation occurred at 48 h. In contrast, the current predation efficiency results under the Ag-Pd BNPs test (1.78 mL/L) showed 100% predation for *Aedes aegypti* with damselfly nymph at 24 h, and for other tests, 100% predation was achieved at 40 h. Therefore, understanding the characteristic and toxic differences between Au-Pd BNPs and Ag-Pd BNPs synthesized using the same reducing agent is crucial for determining their suitability in specific applications. This is especially important given that Au-containing BNPs demonstrate toxicity at higher concentrations, in contrast to Ag-containing BNPs, which exhibit toxicity at lower concentrations.

A pre-requisite to address the potential environmental implications of the synthesized nanoparticles, it is crucial to acknowledge that products incorporating metal nanoparticles, such as silver, titanium, zinc,

gold, magnesium, aluminum oxide, copper, platinum, iron, and iron oxides, are readily accessible in the consumer market. Moreover, the incorporation of these materials into new products is on a continuous rise [83]. The increasing industrial and household applications raise the likelihood of nanoparticles being released into the environment. Consequently, assessing the risk of nanoparticles-mediated ecotoxicity is crucial to formulate specific guidelines for their use, disposal, bioremediation, and implementing strict surveillance for their accidental or intentional environmental release. Despite the numerous benefits, unknown risks may be associated with direct and indirect exposure to nanoparticles. The environmental impact and economic costs associated with the environmental release of nanoparticles are still underdeveloped. The majority of nanoparticle-based products are under study, awaiting industrial-level production, while others are already available in the market [84, 85, 86]. These nanoparticle-based products largely end up in soil, air, and aquatic environments. The nanoparticles contaminate soil, adversely affecting its enzymatic activity, nutritional balance, and self-cleaning properties. This, in turn, has a negative impact on beneficial microbial biodiversity, leading to adverse effects on plant growth and development, as well as bioaccumulation in both edible and non-edible plant tissues. Additionally, the leaching of nanoparticles into nearby aquatic reservoirs further impacts other ecological organisms [87]. Whereas, the optimal use of some metal nanoparticles such as iron and gold in clinical trial studies, specifically for theragnostic and radiotherapy applications, is closely observed, and the final formulation is approved or rejected based on associated implications on human health [88]. However, the unintentional environmental release of nanoparticles from nano-biocomposites used in food packaging technologies is not as closely monitored. This calls for the establishment of standard guidelines to test the ecotoxicological effects of nanoparticles released from packaging materials [89]. The field of environmental nanotechnology actively engages in providing solutions to address the environmental impact, cleanup, and remediation of anthropogenic nanomaterials. Many of these solutions involve the use of mono- and bi-metallic nanoparticles to remove other nano-contaminants, which still pose the risk of contaminating the environment themselves due to high mobility and transport, leading to unintended contamination, creation of toxic byproducts during remediation, and toxicity to local flora and fauna. The short-term stability and persistence of these nanoparticles further pose risks to the environment, leading to bioaccumulation and biomagnification. Additionally, unknown interactions in the environment can lead to the generation of new and harmful compounds. Consequently, bimetallic nanoparticles are observed to play a dual role depending on the mode of application, serving either as a contaminant or as an agent for the remediation of other nano-contaminants [90]. The environmental implications of adverse effects of nanoparticles are high due to their large surface area to volume ratio. Ongoing research on nanoparticles transport, environmental fate, long-term effects, and potential risks associated with their use in remediation, based on the specific characteristics of the nanoparticles, is evident in recent literature. Furthermore, regulatory frameworks are being developed by the U.S., European Union, Canada, Australia, and International Collaborations. These frameworks, such as the Toxic Substances Control Act (TSCA) under the U.S. Environmental Protection Agency (EPA), the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) regulation, Canadian Environmental Protection Act (CEPA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS), and Organization for Economic Co-operation and Development (OECD), respectively, are formulating guidelines for the

responsible use of nanotechnology in environmental applications. This involves collaboration between governments, industry stakeholders, and scientific communities to ensure that regulations are supported by the latest scientific understanding and technological advancements [91, 92, 93].

Lastly, the limitation of the biologically synthesized nanoparticles lies in the use of diverse leaf extracts, each containing a unique phytochemical composition, play a distinctive role in the synthesis of nanoparticles, resulting in variations in the size and shape that subsequently influence their toxicity and other properties. A significant limitation in the available literature lies in the lack of comprehensive comparisons concerning the toxicities observed in bioassays of biologically synthesized nanoparticles. This limitation arises due to the frequent use of units like ppm without disclosing the actual amount of the formulation used for experiments. The common reliance on ppm as a measurement unit, often employed by researchers, fails to provide a clear understanding of the nanoparticles' original specifications employed in the tests. Given the inherent variability in the size and shape of biologically synthesized nanoparticles, the use of ppm becomes less than ideal. A more suitable approach can involve comparing size-related characterizations (SEM, TEM, DLS) with toxicity results presented as raw measurements in units of mL/ng/mg/% of NPs in solvent (units). Additionally, specifying the applied serial dilution of the molar solution during experimentation is imperative.

The current study provides significant information about the novel characteristic features of Ag-Pd BNPs synthesized using a non-toxic *Citrus limon* leaf extract for the first time. It also addresses a critical gap by conducting the first-ever nanotoxicity evaluation of Ag-Pd BNPs against more complex environmental invertebrates. Therefore, moving ahead towards ecotoxicological evaluation compared to the available studies on simpler laboratory based antimicrobial and anticancer assays. In addition to the mosquito larvicidal efficacy of Ag-Pd BNPs, studies against non-target nymphs are required for the selection of the minimal effective concentration. This would help to formulate a sustainable, target-oriented insecticide posing a comparatively lower threat to non-target organisms. Therefore, we recommend that for pest management, simultaneous efficacies against non-target organisms spatially present in the same niche as the target pest should be considered complementary in selecting nanoparticles concentrations. The LC_{50} value varies among environmental organisms and life stages, influenced by their biological complexities. Our findings are consistent with other existing literature, highlighting the potential applicability of *Citrus limon* mediated Ag-Pd BNPs in catalytic, antimicrobial, and anticancer activities.

Conclusion

The findings of this study highlight the successful utilization of a 10% aqueous leaf extract of *Citrus limon* for the synthesis of Ag-Pd BNPs. Characterization studies confirmed the synthesis and physicochemical parameters, consistent with other leaf extract-mediated syntheses. As a result, it is recommended for use in various studies to test its applications, such as for the production of nanosensors, nanocatalysts, and antimicrobial and anti-larvicidal formulations. The Ag-Pd BNPs exhibited toxicity against all larval stages of *Anopheles stephensi* and *Aedes aegypti* mosquitoes. No mortality was observed in non-target organisms at the selected concentration. However, it is advised to

use lower concentrations than those used in the LC_{50} values as observed in the study, for future applications and evaluations to ensure safety against single or multicellular forms of life present in the ecosystem. Considering the potentially toxic nature of Ag-Pd BNPs, unchecked environmental leakage through potential products may result in eco-toxicity and impact spatial fauna. Moreover, their unregulated use and unevaluated toxicity can possibly lead to the extinction of the sensitive species. However, the nanotoxic properties of Ag-Pd BNPs can be sustainably used as a tool against resistant microbial strains and insecticide-resistant vectors and pests, after consideration of recommended safety and bioremediation measures. In the future, bio-insecticidal formulations containing a minimal optimum concentration of Ag-Pd BNPs can be further evaluated for eco-friendly and environmentally conscious applications.

Declarations

Acknowledgments

We sincerely acknowledge our gratitude towards Prof. P. S. Satsangi (Chairman, Advisory Committee on Education), and Prof. P.K. Kalra (Director), Dayalbagh Educational Institute (DEI), Agra. We thank Head, Department of Zoology, DEI, for materials and instruments. We thank Head, Department of Chemistry for, XRD, FTIR spectroscopy facility (for leaf extract), and Zetasizer facility, at the instrumentation lab, DEI. We thank Prof. Tapas Chandra Nag for TEM facility (SAIF, AIIMS, Delhi), Prof. Dinesh Mohan for SEM-EDX spectroscopy facility, AIRF, (JNU, Delhi), and Prof. S. A. Ansari for FTIR and UV-Vis spectroscopy facility (for Ag-Pd BNPs), CIF (Jamia Millia Islamia, Delhi).

Author Contributions Statement

S.P.M. has contributed to the methodology, experimentation, analysis, drafting, and reviewing of the original manuscript.

S.P. has conceptualized the research, provided supervision throughout the study, and contributed to the reviewing of the manuscript.

Competing interests

The authors declare no competing interests.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability

All data generated or analyzed during the study are presented in the supplementary file.

References

1. Das, R. K., Pachapur, V. L., Lonappan, L., Naghdi, M., Pulicharla, R., Maiti, S., ... & Brar, S. K. (2017). Biological synthesis of metallic nanoparticles: plants, animals and microbial aspects. *Nanotechnology for Environmental Engineering*, 2, 1-21.
2. Berta, L., Coman, N. A., Rusu, A., & Tanase, C. (2021). A review on plant-mediated synthesis of bimetallic nanoparticles, characterisation and their biological applications. *Materials*, 14(24), 7677.
3. Fei Guo, C., Sun, T., Cao, F., Liu, Q., & Ren, Z. (2014). Metallic nanostructures for light trapping in energy-harvesting devices. *Light: Science & Applications*, 3(4), e161-e161.
4. Moslah, C., Kandyla, M., Mousdis, G. A., Petropoulou, G., & Ksibi, M. (2018). Photocatalytic properties of titanium dioxide thin films doped with noble metals (Ag, Au, Pd, and Pt). *physica status solidi (a)*, 215(17), 1800023.
5. Narenkumar, J., Parthipan, P., Madhavan, J., Murugan, K., Marpu, S. B., Suresh, A. K., & Rajasekar, A. (2018). Bioengineered silver nanoparticles as potent anti-corrosive inhibitor for mild steel in cooling towers. *Environmental Science and Pollution Research*, 25, 5412-5420.
6. El-Shamy, O. A., & Deyab, M. A. (2023). Eco-friendly biosynthesis of silver nanoparticles and their improvement of anti-corrosion performance in epoxy coatings. *Journal of Molecular Liquids*, 376, 121488.
7. Zhang, J., Ahmadi, M., Fargas, G., Perinka, N., Reguera, J., Lanceros-Méndez, S., ... & Jiménez-Piqué, E. (2022). Silver nanoparticles for conductive inks: From synthesis and ink formulation to their use in printing technologies. *Metals*, 12(2), 234.
8. Mandal, D., Mishra, S., & Singh, R. K. (2018). Green synthesized nanoparticles as potential nanosensors. *Environmental, Chemical and Medical Sensors*, 137-164.,
9. Jebiril, S., Fdhila, A., & Dridi, C. (2021). Nanoengineering of eco-friendly silver nanoparticles using five different plant extracts and development of cost-effective phenol nanosensor. *Scientific Reports*, 11(1), 22060.
10. Rana, A., Yadav, K., & Jagadevan, S. (2020). A comprehensive review on green synthesis of nature-inspired metal nanoparticles: Mechanism, application and toxicity. *Journal of Cleaner Production*, 272, 122880.
11. Mukherjee, S., & Patra, C. R. (2017). Biologically synthesized metal nanoparticles: Recent advancement and future perspectives in cancer theranostics. *Future science OA*, 3(3), FS0203.
12. Kuchur, O. A., Tsymbal, S. A., Shestovskaya, M. V., Serov, N. S., Dukhinova, M. S., & Shtil, A. A. (2020). Metal-derived nanoparticles in tumor theranostics: Potential and limitations. *Journal of inorganic biochemistry*, 209, 111117.
13. Habeeb Rahuman, H. B., Dhandapani, R., Narayanan, S., Palanivel, V., Paramasivam, R., Subbarayalu, R., ... & Muthupandian, S. (2022). Medicinal plants mediated the green synthesis of silver nanoparticles and their biomedical applications. *IET nanobiotechnology*, 16(4), 115-144.
14. Shah, S., Shah, S. A., Faisal, S., Khan, A., Ullah, R., Ali, N., & Bilal, M. (2022). Engineering novel gold nanoparticles using Sageretia thea leaf extract and evaluation of their biological activities. *Journal of Nanostructure in Chemistry*, 12(1), 129-140.

15. Shah, R., Shah, S. A., Shah, S., Faisal, S., & Ullah, F. (2020). Green synthesis and antibacterial activity of gold nanoparticles of *Digera muricata*. *Indian Journal of Pharmaceutical Sciences*, 82(2), 374-378.
16. Ullah, R., Shah, S., Muhammad, Z., Shah, S. A., Faisal, S., Khattak, U., ... & Taj Akbar, M. (2021). In vitro and in vivo applications of *Euphorbia wallichii* shoot extract-mediated gold nanospheres. *Green Processing and Synthesis*, 10(1), 101-111.
17. Faisal, S., Khan, M. A., Jan, H., Shah, S. A., Shah, S., Rizwan, M., & Akbar, M. T. (2020). Edible mushroom (*Flammulina velutipes*) as biosource for silver nanoparticles: from synthesis to diverse biomedical and environmental applications. *Nanotechnology*, 32(6), 065101.
18. Faisal, S., Ullah, R., Alotaibi, A., Zafar, S., Rizwan, M., & Tariq, M. H. (2023). Biofabrication of silver nanoparticles employing biomolecules of *Paraclostridium benzoelyticum* strain: Its characterization and their in-vitro antibacterial, anti-aging, anti-cancer and other biomedical applications. *Microscopy Research and Technique*.
19. Soni, N., & Prakash, S. (2014). Silver nanoparticles: a possibility for malarial and filarial vector control technology. *Parasitology research*, 113, 4015-4022.
20. Verma, A., & Preet, S. (2022). Novel Green Synthesis of Combinational Silver Nanoparticles using *Jatropha integerrima* for Dengue Vector Control and Cytotoxicity Assessment. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 92(4), 747-757.
21. Al-Radadi, N. S., Hussain, T., Faisal, S., & Shah, S. A. R. (2022). Novel biosynthesis, characterization and bio-catalytic potential of green algae (*Spirogyra hyalina*) mediated silver nanomaterials. *Saudi Journal of Biological Sciences*, 29(1), 411-419.
22. Dawadi, S., Katuwal, S., Gupta, A., Lamichhane, U., Thapa, R., Jaisi, S., ... & Parajuli, N. (2021). Current research on silver nanoparticles: Synthesis, characterization, and applications. *Journal of nanomaterials*, 2021, 1-23.
23. Roy, A., Pandit, C., Gacem, A., Alqahtani, M. S., Bilal, M., Islam, S., ... & Jameel, M. (2022). Biologically derived gold nanoparticles and their applications. *Bioinorganic Chemistry and Applications*, 2022.
24. Salayová, A., Bedlovičová, Z., Daneu, N., Baláž, M., Lukáčová Bujňáková, Z., Balážová, Ľ., & Tkáčiková, Ľ. (2021). Green synthesis of silver nanoparticles with antibacterial activity using various medicinal plant extracts: Morphology and antibacterial efficacy. *Nanomaterials*, 11(4), 1005.
25. Borase, H. P., Salunke, B. K., Salunkhe, R. B., Patil, C. D., Hallsworth, J. E., Kim, B. S., & Patil, S. V. (2014). Plant extract: a promising biomatrix for ecofriendly, controlled synthesis of silver nanoparticles. *Applied biochemistry and biotechnology*, 173, 1-29.
26. Rajan, D. S., & Varghese, T. A. (2017). An evaluation on the larvicidal efficacies of aqueous leaf extracts of *Lantana camara* and *Catharanthus roseus* against mosquito larvae. *International Journal of Mosquito Research*, 4(3), 93-97.
27. Vishnukumar, P., Vivekanandhan, S., & Muthuramkumar, S. (2017). Plant-Mediated Biogenic Synthesis of Palladium Nanoparticles: Recent Trends and Emerging Opportunities. *ChemBioEng Reviews*, 4(1), 18-36.

28. Kim, K., Kim, K. L., & Shin, K. S. (2011). Co-reduced Ag/Pd bimetallic nanoparticles: Surface enrichment of Pd revealed by Raman spectroscopy. *The Journal of Physical Chemistry C*, 115(30), 14844-14851.
29. Wong, S. T., Shamsuddin, M., Alizadeh, A., & Yun, Y. S. (2016). In-situ generated palladium seeds lead to single-step bioinspired growth of AuPd bimetallic nanoparticles with catalytic performance. *Materials Chemistry and Physics*, 183, 356-365.
30. Minal, S. P., & Prakash, S. (2020). Laboratory analysis of Au–Pd bimetallic nanoparticles synthesized with Citrus limon leaf extract and its efficacy on mosquito larvae and non-target organisms. *Scientific reports*, 10(1), 21610.
31. Mohan, S., Devan, M. V., Sambathkumar, S., Shanmugam, V., Ravikumar, K., Marnadu, R., ... & Hegazy, H. H. (2021). Dual probes of Ag/Pd bimetallic NPs facilely synthesized by green process using Catharanthus leaf extract on textile dye removal and free radical capability. *Applied Nanoscience*, 11, 1565-1574.
32. Lu, F., Sun, D., Huang, J., Du, M., Yang, F., Chen, H., ... & Li, Q. (2014). Plant-mediated synthesis of Ag–Pd alloy nanoparticles and their application as catalyst toward selective hydrogenation. *ACS Sustainable Chemistry & Engineering*, 2(5), 1212-1218.
33. Turunc, E., Binzet, R., Gumus, I., Binzet, G., & Arslan, H. (2017). Green synthesis of silver and palladium nanoparticles using *Lithodora hispidula* (Sm.) Griseb.(Boraginaceae) and application to the electrocatalytic reduction of hydrogen peroxide. *Materials Chemistry and Physics*, 202, 310-319.
34. Velpula, S., Beedu, S. R., & Rupula, K. (2021). Bimetallic nanocomposite (Ag-Au, Ag-Pd, Au-Pd) synthesis using gum kondagogu a natural biopolymer and their catalytic potentials in the degradation of 4-nitrophenol. *International Journal of Biological Macromolecules*, 190, 159-169.
35. Abdel-Fattah, W. I., Eid, M. M., Abd El-Moez, S. I., Mohamed, E., & Ali, G. W. (2017). Synthesis of biogenic Ag@ Pd Core-shell nanoparticles having anticancer/antimicrobial functions. *Life sciences*, 183, 28-36.
36. Sivamaruthi, B. S., Ramkumar, V. S., Archunan, G., Chaiyasut, C., & Suganthi, N. (2019). Biogenic synthesis of silver palladium bimetallic nanoparticles from fruit extract of *Terminalia chebula*–In vitro evaluation of anticancer and antimicrobial activity. *Journal of Drug Delivery Science and Technology*, 51, 139-151.
37. Singh, P., Halder, M., Ray, S., Bose, A., & Sen, K. (2020). Green synthesis of silver and palladium nanocomposites: a study of catalytic activity towards etherification reaction. *Materials Advances*, 1(8), 2937-2952.
38. Li, C., Han, Y., Gao, T., Zhang, J., Xu, D. X., & Wāng, Y. (2023). Insecticidal activity of metallic nanopesticides synthesized from natural resources: A review. *Environmental Chemistry Letters*, 21(2), 1141-1176.
39. Onen, H., Luzala, M. M., Kigozi, S., Sikumbili, R. M., Muanga, C. J. K., Zola, E. N., ... & Memvanga, P. B. (2023). Mosquito-Borne Diseases and Their Control Strategies: An Overview Focused on Green Synthesized Plant-Based Metallic Nanoparticles. *Insects*, 14(3), 221.

40. Pouthika, K., & Madhumitha, G. (2023). A review on plant-derived nanomaterials: an effective and innovative insect-resistant strategy for alternate pesticide development. *International Journal of Environmental Science and Technology*, 1-24.
41. Nie, D., Li, J., Xie, Q., Ai, L., Zhu, C., Wu, Y., ... & Tan, W. (2023). Nanoparticles: A Potential and Effective Method to Control Insect-Borne Diseases. *Bioinorganic Chemistry and Applications*, 2023.
42. Minal, S. P., & Prakash, S. Cu–Zn and Ag–Cu bimetallic nanoparticles as larvicide to control malaria parasite vector: a comparative analysis. In 2016 IEEE region 10 Humanitarian technology conference (R10-HTC) (1–6) (IEEE, 2016).
43. Minal, S. P., & Prakash, S. (2018). Characterization and nano-efficacy study of palladium nanoparticles against Larvae of *Anopheles stephensi* (Liston). *International Journal of Advanced Engineering and Nanotechnology*, 3(10), 1-5.
44. Minal, P. S., & Prakash, S. (2019). Efficacy of bimetallic copper-zinc nanoparticles against larvae of microfilariae vector in laboratory. *International Journal of Scientific Research*, 8(2), 1-4.
45. World malaria report 2022 <https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022>
46. Tolle, M. A. (2009). Mosquito-borne diseases. *Current problems in pediatric and adolescent health care*, 39(4), 97-140.
47. Vector-borne diseases - World Health Organization (WHO) (<https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases>)
48. Bruguera, S., Fernández-Martínez, B., Martínez-de la Puente, J., Figuerola, J., Porro, T. M., Rius, C., ... & Gomez-Barroso, D. (2020). Environmental drivers, climate change and emergent diseases transmitted by mosquitoes and their vectors in southern Europe: A systematic review. *Environmental research*, 191, 110038.
49. Filho, W. L., Scheday, S., Boenecke, J., Gogoi, A., Maharaj, A., & Korovou, S. (2019). Climate change, health and mosquito-borne diseases: Trends and implications to the pacific region. *International journal of environmental research and public health*, 16(24), 5114.
50. Franklin, L. H., Jones, K. E., Redding, D. W., & Abubakar, I. (2019). The effect of global change on mosquito-borne disease. *The Lancet infectious diseases*, 19(9), e302-e312.
51. Rani, A., Gupta, A., Nagpal, B. N., Gupta, S. K., Prasad, P., & Singh, H. (2023). Impact of urbanization on the abundance and distribution of Anophelines population in Ghaziabad district, Uttar Pradesh, India. *Journal of Vector Borne Diseases*.
52. World Health Organization, & UNICEF. (2017). *Global vector control response 2017-2030*.
53. Govindarajan, M., Rajeswary, M., Muthukumaran, U., Hoti, S. L., Khater, H. F., & Benelli, G. (2016). Single-step biosynthesis and characterization of silver nanoparticles using *Zornia diphylla* leaves: A potent eco-friendly tool against malaria and arbovirus vectors. *Journal of Photochemistry and Photobiology B: Biology*, 161, 482-489.
54. Govindarajan, M., Khater, H. F., Panneerselvam, C., & Benelli, G. (2016). One-pot fabrication of silver nanocrystals using *Nicandra physalodes*: A novel route for mosquito vector control with moderate

- toxicity on non-target water bugs. *Research in Veterinary Science*, 107, 95-101.
55. Foko, L. P. K., Meva, F. E. A., Moukoko, C. E. E., Ntomba, A. A., Ekoko, W. E., Belle, P. E. K., ... & Lehman, L. G. (2021). Green-synthesized metal nanoparticles for mosquito control: A systematic review about their toxicity on non-target organisms. *Acta Tropica*, 214, 105792.
 56. WRBU: Mosquito Identification Resources http://www.wrbu.org/VecID_MQ.html
 57. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides (No. WHO/CDS/WHOPES/GCDPP/2005.13). World Health Organization (2005).
 58. Odonata | Species | India Biodiversity Portal *Bradinopyga geminata* (Rambur, 1842) <https://india.biodiversity.org/species/show/226725?pos=>
 59. Odonata | Species | India Biodiversity Portal *Ischnura senegalensis* (Rambur, 1842) <https://indiabiodiversity.org/species/show/234358?pos=>
 60. Saha, N., Aditya, G., Banerjee, S. & Saha, G. K. Predation potential of odonates on mosquito larvae: implications for biological control. *Biol. Control* 63(1), 1–8 (2012).
 61. Murugan, K., Nataraj, D., Madhiyazhagan, P., Sujitha, V., Chandramohan, B., Panneerselvam, C., ... & Benelli, G. (2016a). Carbon and silver nanoparticles in the fight against the filariasis vector *Culex quinquefasciatus*: genotoxicity and impact on behavioral traits of non-target aquatic organisms. *Parasitology research*, 115, 1071-1083.
 62. Ferreira, T., & Rasband, W. (2012). ImageJ user guide. *ImageJ/Fiji*, 1, 155-161.
 63. Abbott, W. S. A method of computing the effectiveness of insecticides. *J. Ecol. Entomol.* 18, 265–267 (1925).
 64. Finney, D. J. & Stevens, W. L. A table for the calculation of working probits and weights in probit analysis. *Biometrika* 35(1–2), 191–201 (1948)
 65. Finney, D. J. (ed.) *Probit Analysis* (Cambridge University Press, Cambridge, 1952).
 66. Coates, J. (2000). Interpretation of infrared spectra, a practical approach. *Encyclopedia of Analytical Chemistry*, 12, 10815-10837.
 67. Origin(Pro), 2023b. OriginLab Corporation, Northampton, MA, USA.
 68. Yeap, S. P., Lim, J., Ngang, H. P., Ooi, B. S., & Ahmad, A. L. (2018). Role of particle–particle interaction towards effective interpretation of Z-average and particle size distributions from dynamic light scattering (DLS) analysis. *Journal of nanoscience and nanotechnology*, 18(10), 6957-6964.
 69. Souza, T. G., Ciminelli, V. S., & Mohallem, N. D. S. (2016, July). A comparison of TEM and DLS methods to characterize size distribution of ceramic nanoparticles. In *Journal of Physics: Conference Series* (Vol. 733, No. 1, p. 012039). IOP Publishing.
 70. Bhattacharjee, S. (2016). DLS and zeta potential—what they are and what they are not?. *Journal of controlled release*, 235, 337-351.
 71. Soni, N., & Prakash, S. (2012). Entomopathogenic fungus generated Nanoparticles for enhancement of efficacy in *Culex quinquefasciatus* and *Anopheles stephensi*. *Asian Pacific Journal of Tropical Disease*, 2, S356-S361.

72. Bhakayaraj, K., Kumaraguru, S., Gopinath, K., Sabitha, V., Kaleeswarran, P. R., Karthika, V., ... & Arumugam, A. (2017). Eco-friendly synthesis of palladium nanoparticles using *Melia azedarach* leaf extract and their evaluation for antimicrobial and larvicidal activities. *Journal of Cluster Science*, 28, 463-476.
73. Kalimuthu, K., Panneerselvam, C., Chou, C., Tseng, L. C., Murugan, K., Tsai, K. H., ... & Benelli, G. (2017). Control of dengue and Zika virus vector *Aedes aegypti* using the predatory copepod *Megacyclops formosanus*: synergy with *Hedychium coronarium*-synthesized silver nanoparticles and related histological changes in targeted mosquitoes. *Process Safety and Environmental Protection*, 109, 82-96.
74. Fouad, H., Hongjie, L., Hosni, D., Wei, J., Abbas, G., Ga'al, H., & Jianchu, M. (2018). Controlling *Aedes albopictus* and *Culex pipiens pallens* using silver nanoparticles synthesized from aqueous extract of *Cassia fistula* fruit pulp and its mode of action. *Artificial cells, nanomedicine, and biotechnology*, 46(3), 558-567.
75. Ga'al, H., Fouad, H., Tian, J., Hu, Y., Abbas, G., & Mo, J. (2018). Synthesis, characterization and efficacy of silver nanoparticles against *Aedes albopictus* larvae and pupae. *Pesticide biochemistry and physiology*, 144, 49-56.
76. dos Santos Lima, J. C., Moreira, R. A., Neto, A. J. G., de Pádua Andrade, D., Freitas, E. C., Daam, M. A., & Rocha, O. (2021). Metal Toxicity Can Affect Dragonfly Nymphs and Ostracods Predation Rates and Food Selectivity: Ecological Implications on Food Webs. *Water, Air, & Soil Pollution*, 232, 1-12.
77. Azam, I., Afsheen, S., Zia, A., Javed, M., Saeed, R., Sarwar, M. K., & Munir, B. (2015). Evaluating insects as bioindicators of heavy metal contamination and accumulation near industrial area of Gujrat, Pakistan. *BioMed research international*, 2015.
78. Akhtar, Z. R., Tariq, K., Mavian, C., Ali, A., Ullah, F., Zang, L. S., ... & Ali, S. (2021). Trophic transfer and toxicity of heavy metals from dengue mosquito *Aedes aegypti* to predator dragonfly *Tamea cophysa*. *Ecotoxicology*, 30(6), 1108-1115.
79. Murugan, K., Dinesh, D., Kumar, P. J., Panneerselvam, C., Subramaniam, J., Madhiyazhagan, P., ... & Benelli, G. (2015a). *Datura metel*-synthesized silver nanoparticles magnify predation of dragonfly nymphs against the malaria vector *Anopheles stephensi*. *Parasitology research*, 114, 4645-4654.
80. Murugan, K., Benelli, G., Ayyappan, S., Dinesh, D., Panneerselvam, C., Nicoletti, M., ... & Suresh, U. (2015b). Toxicity of seaweed-synthesized silver nanoparticles against the filariasis vector *Culex quinquefasciatus* and its impact on predation efficiency of the cyclopoid crustacean *Mesocyclops longisetus*. *Parasitology research*, 114, 2243-2253.
81. Murugan, K., Panneerselvam, C., Subramaniam, J., Madhiyazhagan, P., Hwang, J. S., Wang, L., ... & Benelli, G. (2016b). Eco-friendly drugs from the marine environment: spongweed-synthesized silver nanoparticles are highly effective on *Plasmodium falciparum* and its vector *Anopheles stephensi*, with little non-target effects on predatory copepods. *Environmental Science and Pollution Research*, 23, 16671-16685.

82. Kalimuthu, K., Panneerselvam, C., Chou, C., Lin, S. M., Tseng, L. C., Tsai, K. H., ... & Hwang, J. S. (2017). Predatory efficiency of the copepod *Megacyclops formosanus* and toxic effect of the red alga *Gracilaria firma*-synthesized silver nanoparticles against the dengue vector *Aedes aegypti*. *Hydrobiologia*, 785, 359-372.
83. Vance, M. E., Kuiken, T., Vejerano, E. P., McGinnis, S. P., Hochella Jr, M. F., Rejeski, D., & Hull, M. S. (2015). Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. *Beilstein journal of nanotechnology*, 6(1), 1769-1780.
84. Stark, Wendelin J., et al. "Industrial applications of nanoparticles." *Chemical Society Reviews* 44.16 (2015): 5793-5805.
85. Nowack, B., & Bucheli, T. D. (2007). Occurrence, behavior and effects of nanoparticles in the environment. *Environmental pollution*, 150(1), 5-22.
86. Taghavi, S. M., Momenpour, M., Azarian, M., Ahmadian, M., Souiri, F., Taghavi, S. A., ... & Karchani, M. (2013). Effects of nanoparticles on the environment and outdoor workplaces. *Electronic physician*, 5(4), 706.
87. Rajput, V. D., Minkina, T., Sushkova, S., Tsitsuashvili, V., Mandzhieva, S., Gorovtsov, A., ... & Gromakova, N. (2018). Effect of nanoparticles on crops and soil microbial communities. *Journal of Soils and Sediments*, 18, 2179-2187.
88. Bobo, D., Robinson, K. J., Islam, J., Thurecht, K. J., & Corrie, S. R. (2016). Nanoparticle-based medicines: a review of FDA-approved materials and clinical trials to date. *Pharmaceutical research*, 33, 2373-2387.
89. Adeyemi, J. O., & Fawole, O. A. (2023). Metal-based nanoparticles in food packaging and coating technologies: A review. *Biomolecules*, 13(7), 1092.
90. Patil, S. S., Shedbalkar, U. U., Truskewycz, A., Chopade, B. A., & Ball, A. S. (2016). Nanoparticles for environmental clean-up: a review of potential risks and emerging solutions. *Environmental Technology & Innovation*, 5, 10-21.
91. Ali, F., Neha, K., & Parveen, S. (2022). Current regulatory landscape of nanomaterials and nanomedicines: A global perspective. *Journal of Drug Delivery Science and Technology*, 104118.
92. Allan, J., Belz, S., Hoeveler, A., Hugas, M., Okuda, H., Patri, A., ... & Anklaam, E. (2021). Regulatory landscape of nanotechnology and nanoplastics from a global perspective. *Regulatory Toxicology and Pharmacology*, 122, 104885.
93. Mohammadi, Z., & Jafari, S. M. (2021). Regulatory principles on food nanoparticles legislated by Asian and Oceanian countries. In *Safety and Regulatory Issues of Nanoencapsulated Food Ingredients* (pp. 201-238). Academic Press.

Figures

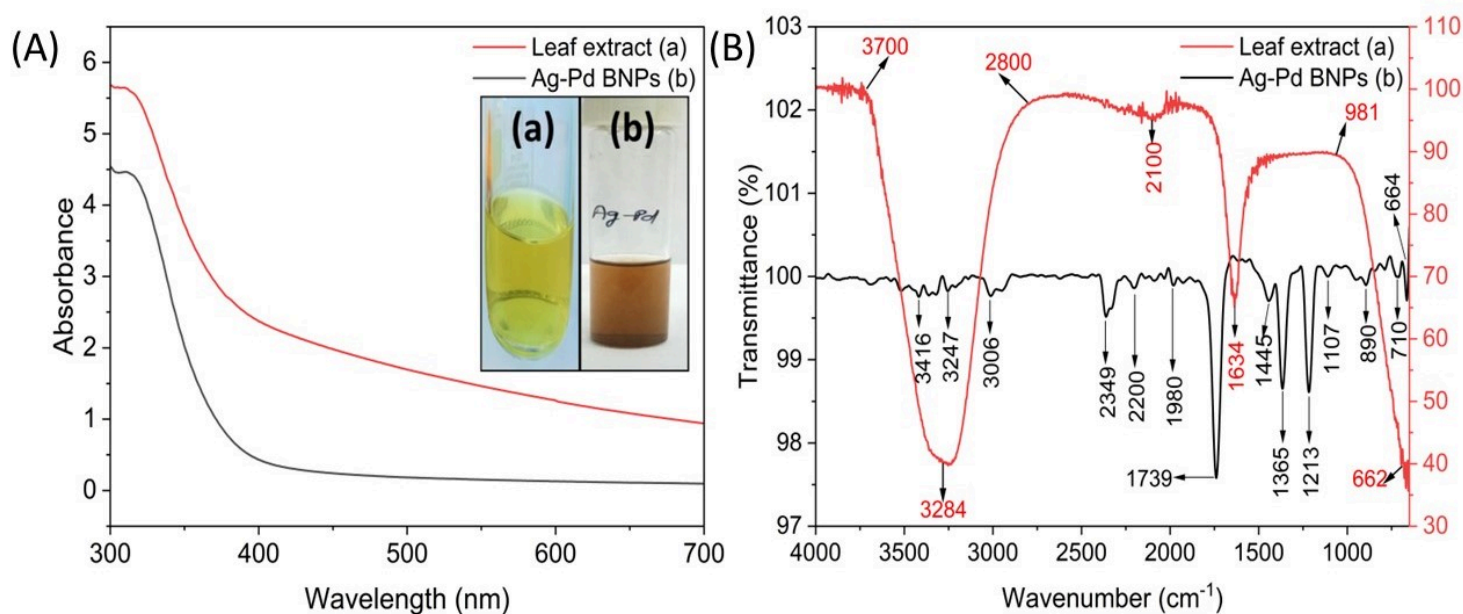


Figure 1

(A) UV VIS absorption spectra; and (B) FT-IR transmittance spectra, for: (a) 10% aqueous Leaf extract of plant *Citrus limon* (1:9) [inset A (a)], (b) diluted sample of Ag-Pd BNPs (1:10) [Inset A (b)].

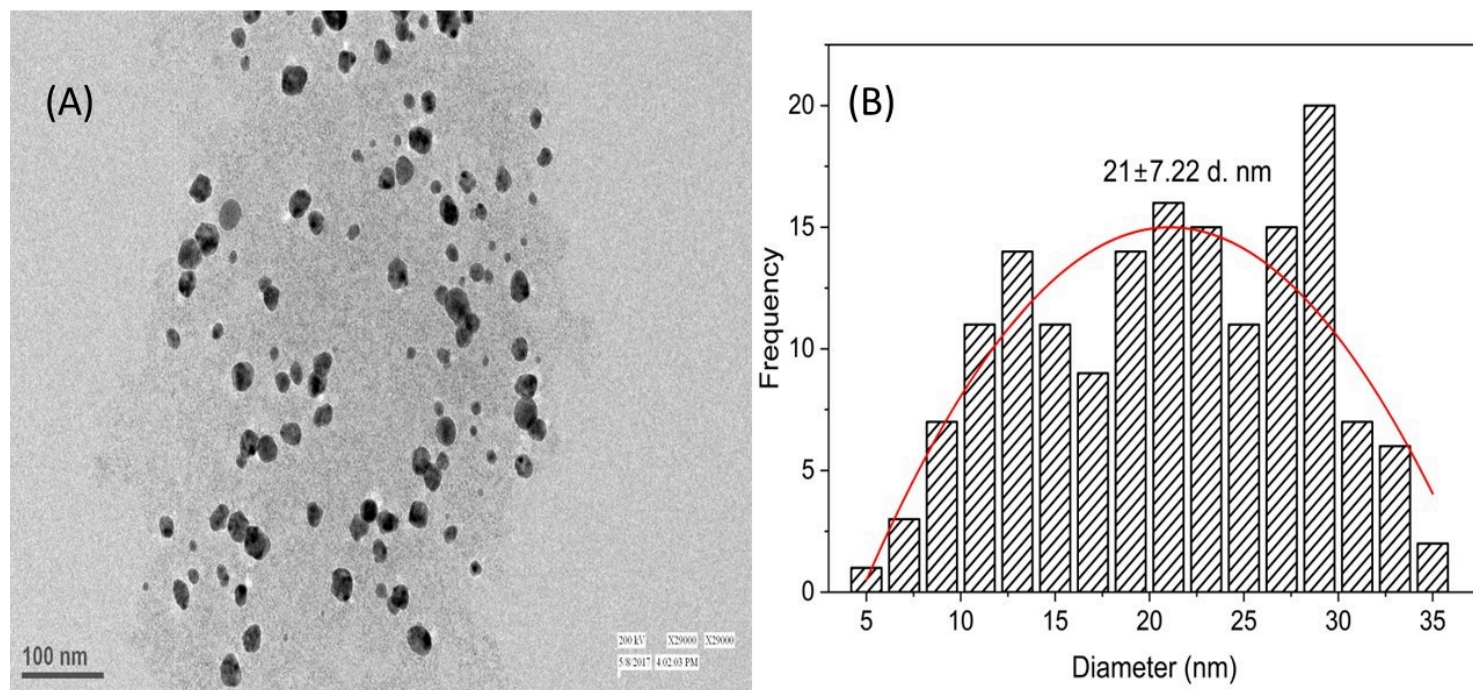


Figure 2

(A) TEM micrograph of Ag-Pd BNPs (1:10), and (B) Particle size distribution histogram with Gaussian fit obtained from the ImageJ analysis of the TEM micrograph (A)

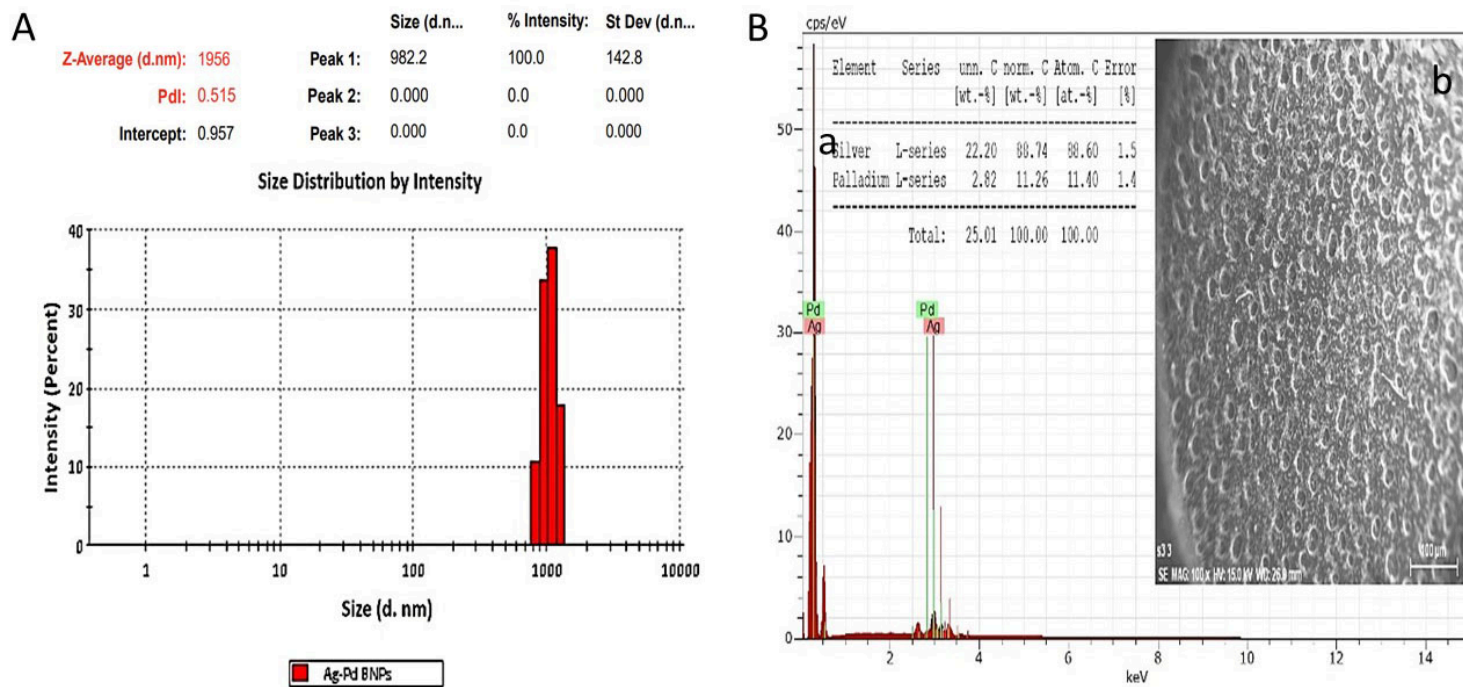


Figure 3

(A) DLS of Ag-Pd BNPs (1:10), and (B) XRD pattern generated for Ag-Pd BNPs sample

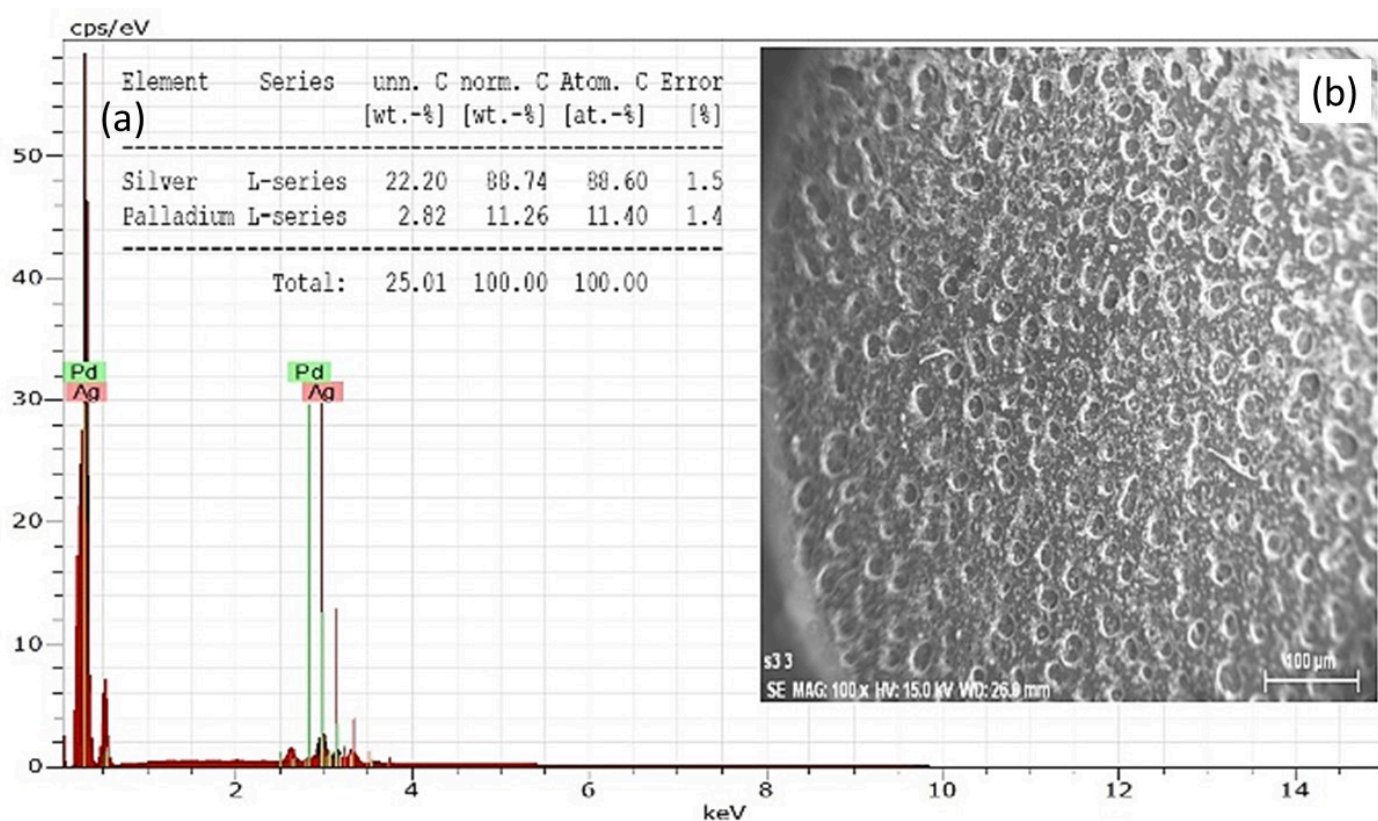


Figure 4

SEM-EDX Analysis [inset: (a) EDX table for elements analysis result of the Ag-Pd BNPs samples shown in SEM micrograph (b)]

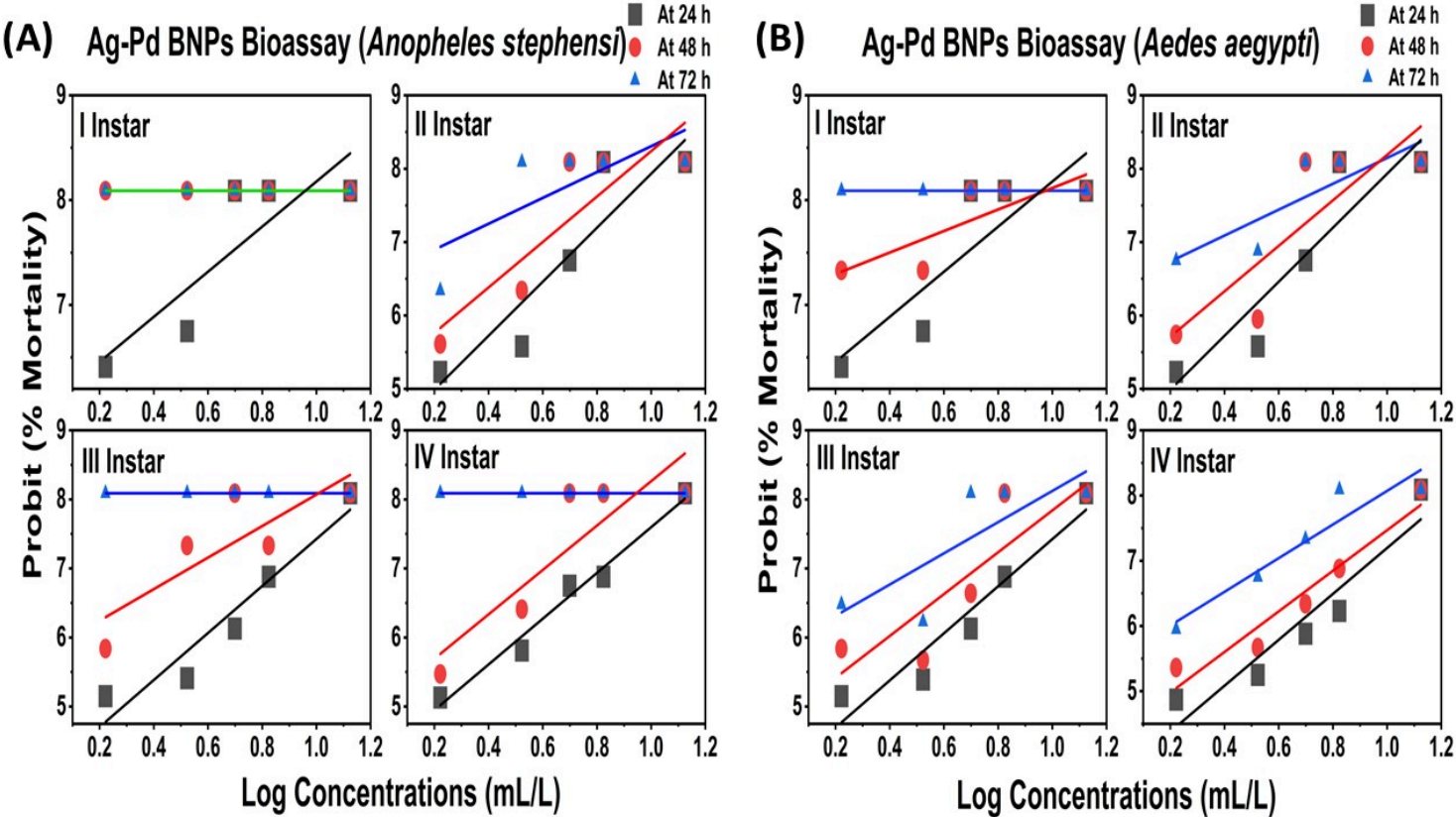


Figure 5

Probit Analysis: Regression analysis to determine LC_{50} from the Ag-Pd BNPs bioassay against I-IV instar larvae of: (A) *Anopheles stephensi* (B) *Aedes aegypti*, at 24 h, 48 h, and 72 h of exposure.

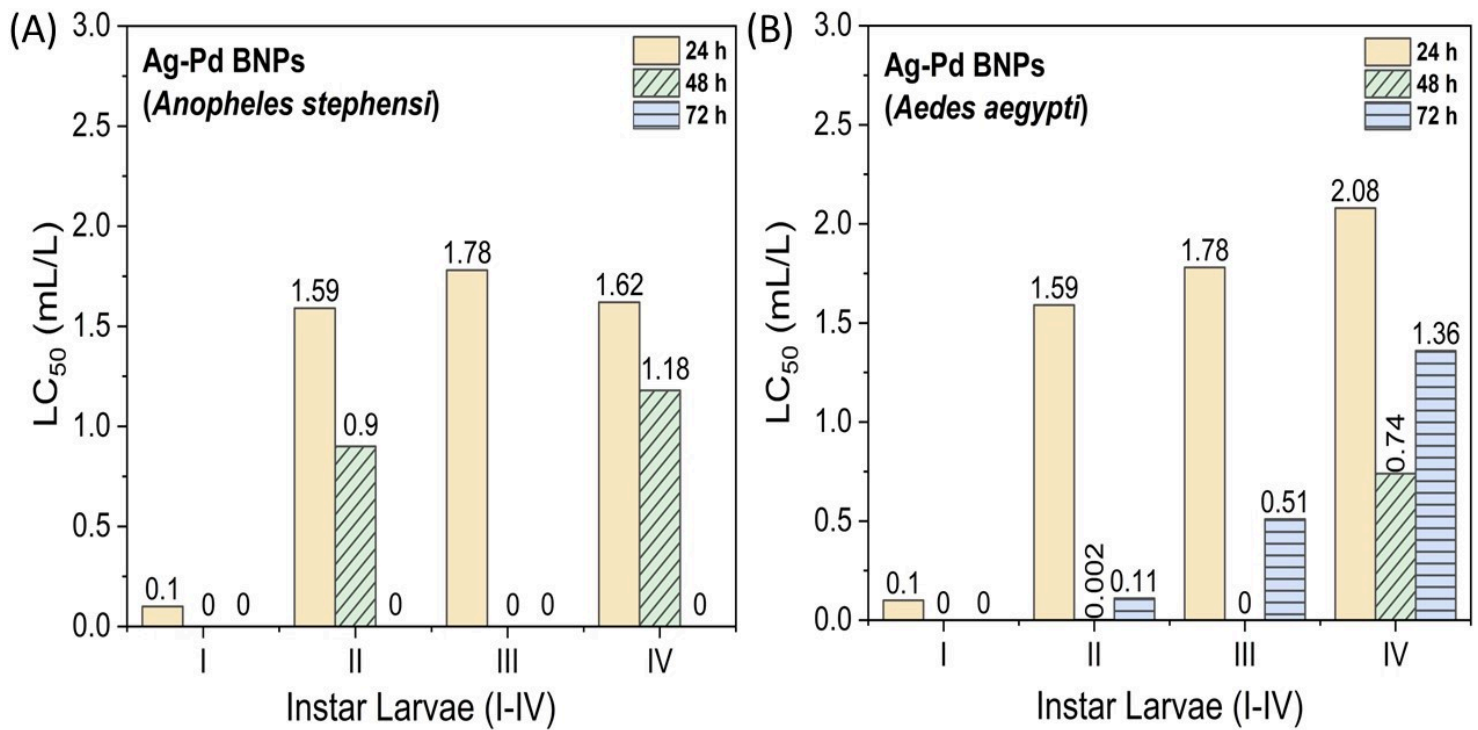


Figure 6

Comparative LC₅₀ value determined from the Ag-Pd BNPs bioassay against I-IV instar larvae of: (A) *Anopheles stephensi* (B) *Aedes aegypti*, at 24 h, 48 h, and 72 h of exposure.

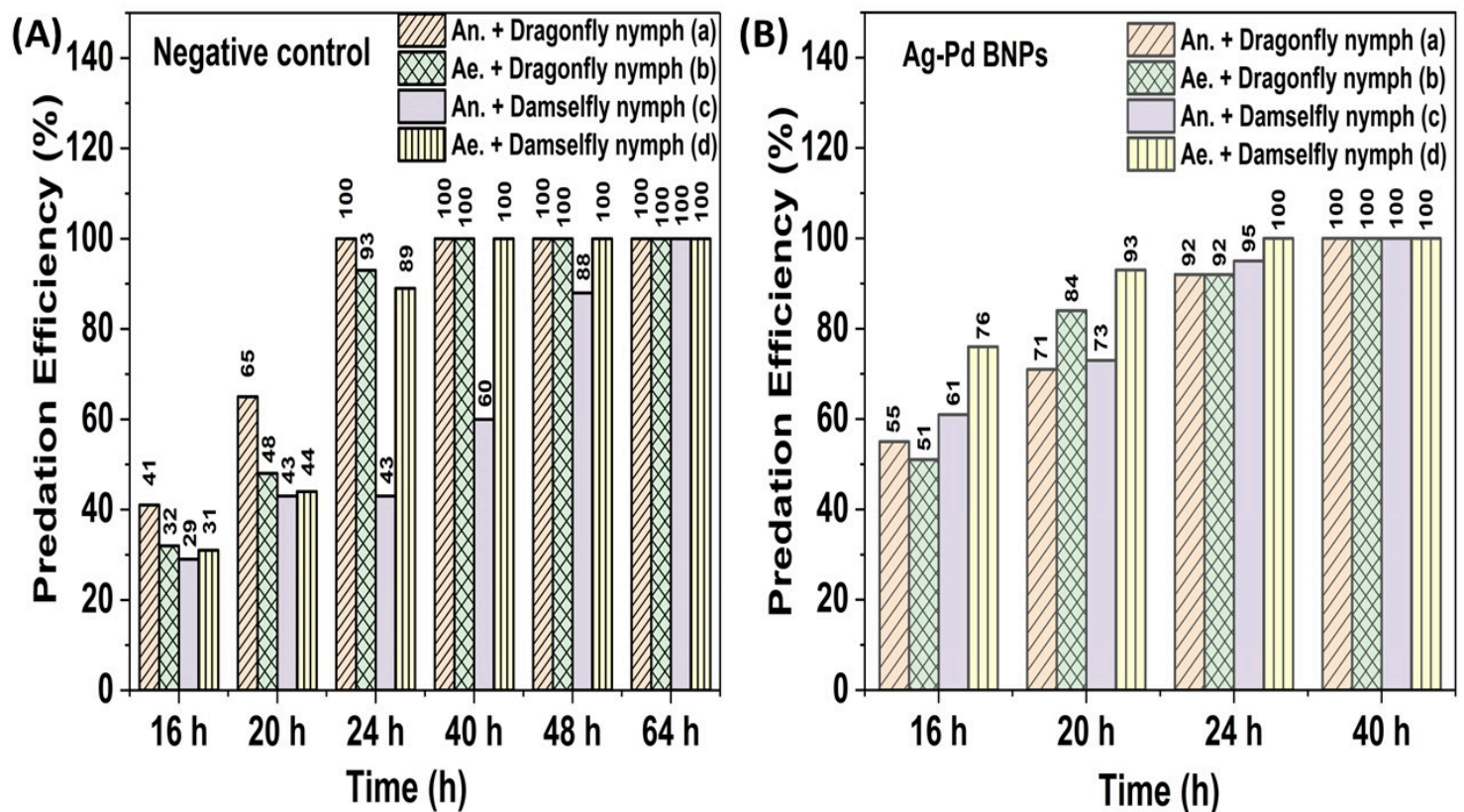


Figure 7

Predation efficiency test of dragonfly (a, c) and damselfly (b, d) in (A) Negative Control, and (B) test concentration containing selected LC_{50} concentration of Ag-Pd BNPs, against the III instar larvae of both *Anopheles stephensi* (An.) *Aedes aegypti* (Ae.)

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