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Anti-cockroach activities of biosynthesized silver nanoparticles using *Petiveria alliacea* extracts

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Abstract

Background This study was conducted with a view to biosynthesize silver nanoparticles (AgNPs) using the root (PaR) and leaf (PaL) extracts of *Petiveria alliacea* and evaluation of their activities against cockroaches, which is a major household insect pest.

Methods The biosynthesized AgNPs were characterized by UV–Vis spectrophotometry, Fourier transform infrared (FTIR) spectroscopy, transmission electron microscopy (TEM), and energy-dispersive X-ray (EDX). PaR-AgNPs and PaL-AgNPs were tested at three different concentrations (1, 5, 10% v/v) using two modes of applications (fumigant and contact toxicity) at different hours of exposure.

Results The peak absorbance of the AgNPs occurred at the wavelengths of 426 and 442 nm for PaL-AgNPs and PaR-AgNPs, respectively, with the brownish colloidal solutions. FITR peaks indicated the presence of alkenes (=C–H), esters (=O), amides (=N–H), and aliphatic amines (=C–N) in the samples, while the TEM and EDX analyses showed the presence of silver in the AgNPs colloidal solution, with the spherical nanoparticles ranging from 5.95 to 76.19 nm in size. Both AgNPs demonstrated insecticidal action against adult cockroaches, with the fumigant application causing more rapid death than the contact application. The fumigant application resulted in 80–90% cockroach mortality, while the contact application caused 40–50% mortality after three hours of exposure at 10% v/v. These results demonstrate that the effectiveness of the bio-fabricated AgNP formulations depends on the concentrations, hours of exposure, and mode of application.

Conclusion Therefore, the pest management program for household pests, particularly cockroaches, can incorporate silver nanoparticle-based insecticides.

Article Highlights
- TEM and EDX analyses indicate the presence of silver in the nano-insecticide products
- Biosynthesized AgNPs exhibited insecticidal action against adult cockroach
- Toxicity effect of nano-insecticides depends on mode of application
- Nano-insecticides applied through fumigant killed faster than when applied through contact
- Effectiveness of biosynthesized AgNPs was dose-dependent

Keywords Cockroach, *Petiveria alliacea*, Silver nanoparticles, Biosynthesis, Nano-insecticide, Fumigant, Contact insecticide

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1 Background

Globally, the cockroach is a prominent household insect [1]. *Periplaneta americana* (L.) stands out as one of the six predominant cockroach species in Nigeria, particularly in the southwestern region. This is frequently observed in residential structures, particularly in the kitchen and bathroom, as well as in restaurants, hotels, and bakeries [2]. The presence of cockroaches poses significant health risks due to their potential to transmit diseases through contact with human food [2, 3]. *P. americana* serves as a coprophagous vector, contaminating significant amounts of consumables by depositing human fecal matter along with parasitic cysts and eggs of helminths and protozoa [2]. Ruhma et al. [4] indicate that infestations of cockroaches can lead to allergic reactions in certain individuals, which may manifest as asthma and skin irritation [5].

Considering the issues outlined, the application of synthetic insecticides has emerged as the predominant approach to mitigate infestations due to their rapid effectiveness against cockroaches. The primary categories of synthetic insecticides include organochlorines, organophosphates, carbamates, and pyrethroids [6, 7]. The stability of these classes of insecticides in the environment raises significant concerns regarding potential environmental and health risks [8, 9]. Previous research indicates that certain species of cockroaches have acquired resistance to synthetic insecticides [10, 11]. The availability of these insecticides, which is critical, has emerged as a significant concern, especially in light of their rising costs [12, 13]. The various issues linked to synthetic insecticides highlight the need for alternatives, particularly biopesticides and nanopesticides.

Biopesticides are characterized as biological pesticides that can be derived from plants, microorganisms, and animals [14, 15]. These substances are characterized as naturally occurring chemicals derived from organic sources, serving as alternatives to synthetic compounds in pest management [16, 17]. Biopesticides are eco-friendly, can be produced easily and at a low cost, and are effective in targeting and eliminating pests [18–20]. A nanopesticide is classified as a biopesticide because of its inherent properties associated with biopesticides [14]. Nanoparticles in the formulations of nano-insecticides can function as adjuvants, thereby enhancing the efficacy of insecticides [15]. The formulation of nano-insecticides can involve a combination of metals and other diverse materials that have been proven effective against specific pests [21, 22].

P. alliacea is a member of the Phytolaccaceae family, which is prevalent in the tropical regions of South and Central America, Africa, and the Caribbean [23]. A variety of chemical compounds have been isolated from *P. alliacea*, including astilbin, myricitrin, engeletin (flavonoids), triterpenes, daucosterol, and lignoceric acid [23]. In the meantime, dibenzyl trisulfide and dichloromethane are among the chemical compounds that exhibit pharmacological and insecticidal properties [23–26]. Nonetheless, there is a lack of information regarding the production and evaluation of nano-insecticides derived from *P. alliacea*, and this represents the inaugural report of Pa-AgNPs in the management of cockroach populations.

2 Methods

2.1 Materials

Leaf and root extracts of *P. alliacea,* silver nitrate, filter paper, insect box, syringe, cotton wool, buckets, transparent containers, paper tape, biscuits, pineapple, and insect-rearing wooden cage.

2.2 Test insect collection

The cockroaches (*P. americana*) were collected from slums, dumping grounds, and several households in Ogbomoso city in Oyo State, southwestern Nigeria, between April 2021 and July 2021. They were introduced into insect wooden cage boxes (20 cm × 15 cm × 5 cm), and rags of different sizes were placed into each box to help mimic the natural environment of the cockroach. Feed consisted of biscuits and pineapple juice, which were evenly mixed together, and these were given as feed. Each wooden cage consisted of 25 adult, unsexed cockroaches, and they were allowed to acclimatize to their new environment for a month. Healthy cockroaches were picked from the insect box irrespective of the gender and used for the study.

2.3 Biosynthesis and characterization of silver nanoparticles

Leaves and roots of *P. alliacea* (Voucher No: LHO 904) were harvested and air-dried under laboratory conditions for several weeks. The dried plant parts were blended separately using an electronic grinding machine (Silver Crest 50 watts) into fine particles.

Silver nanoparticles: Nano *P. alliacea* leaf (PaL-AgNPs) and Nano *P. alliacea* root (PaR-AgNPs) used as nano-insecticides in this study were synthesized using both leaf and root extracts of *P. alliacea* following the modified methods of Lateef et al. [27]. Extracts were prepared by heating 5 g of the powdered sample (leaf or root of *P. alliacea*) dissolved in 100 ml of distilled water at 60 °C for 1 h, then filtered through Whatman No. 1 filter paper, and the filtrate was subsequently centrifuged at 4000 rpm for 30 min to obtain the respective extract.

The biosynthesis was then carried out by reacting about 2 ml of aqueous extract with 40 ml of 1 mM silver nitrate ($AgNO_3$) solution for the synthesis of AgNPs at ambient temperature (30 ± 2 °C) under bright light conditions. The biosynthesis was monitored by observing the change of color of the solution.

Moreover, characterization of the biosynthesized AgNPs was carried out by measuring the absorbance spectrum using a UV–Vis spectrophotometer. Fourier transform infrared (FTIR) spectroscopy analysis was done to establish the biomolecules responsible for the synthesis, capping, and stabilization of AgNPs. As described by Lateef et al. [27], the AgNPs were further observed on the transmission electron microscope (TEM 1400, JEOL, USA) to determine the size and morphology couple with the selected area electron diffraction (SAED).

2.4 Bioassay

2.4.1 Contact toxicity bioassay

This experiment consisted of eight units made up of four healthy adult unsexed cockroaches picked from the insect wooden cage box. It commenced in the morning at 8 am. From the stock solution, 1, 5, and 10% v/v were prepared by diluting the equivalent volume of the stock solution with 100 ml of water, and 5 ml of each tested concentration was applied to the studied insect. The study used a calibrated syringe to measure 5 ml of the prepared concentrations (1, 5, and 10% v/v) of PaL-AgNPs and PaR-AgNPs formulations. Each experimental unit was well labeled with the corresponding treatment names, viz., PaL-AgNPs, PaR-AgNPs, synthetic insecticide (lambdacyhalothrin), and control (water). Diluted lambdacyhalothrin (1 ml of lambdacyhalothrin into 2000 ml of water) was applied as a positive control. The solution drawn with the medical syringe (5 ml) was sprayed directly on the cockroaches in each designated experimental unit. Each treatment was replicated three times, and mortality was recorded at an hourly interval for 20 h. Each of the treatments was replicated three times.

2.4.2 Fumigant toxicity bioassay

The transparent containers (8 units) used for each experimental unit were weighed with a sensitive scale, and 0.1 g of cotton wool was placed into each of the six containers used for this experiment. Then, 5 ml of the tested concentrations (1, 5, and 10% v/v) of Nano *P. alliacea* solution was applied to the cotton wool (8) in each designated container separately. Water was applied as a negative control to the studied insect, while diluted lambdacyhalothrin, as previously stated, was applied as a positive control. The four active adult unsexed cockroaches were then introduced into each assigned container, and mortality was recorded every hour for 20 h. The experiment was replicated three times for each treatment.

2.5 Data collection and statistical analysis

The mortality of the studied insect was determined and later subjected to percentage mortality using Abbot's formula (1925):

$$\%\text{Mortality} = \frac{\text{CA} - \text{TA}}{\text{CA}} \times 100$$

where, TA is the number of dead treated cockroach; CA is the number of introduced cockroach.

While the percentage mortality was corrected using Abbot's formula (1925):

$$\text{Pr} = \frac{\text{Pa} - \text{Pc}}{100 - \text{Pc}} \times 100$$

where, Pr is the percentage of efficacy of the insecticide, Pa is the percentage mortality of the treated insect, and Pc is the percentage mortality of the untreated insect.

Percentage mortalities were later subjected to analysis of variance (ANOVA) using statistical software analysis (2005) version 2.0. Means were separated using least significant difference (LSD) at 5% probability.

3 Results

3.1 Biosynthesis and characterization of AgNPs

An extract of the leaves and roots of *P. alliacea* plant made AgNPs (Nano *P. alliacea* leaf (PaL-AgNPs) and Nano *P. alliacea* root (PaR-AgNPs) in 10 min at room temperature (30 ± 2 °C), with PaL-AgNPs being a dark brown color and PaR-AgNPs being a light brown color (Fig. 1). The colors got darker over time and finally became stable after 25 min. Moreover, maximum absorbance occurred at the wavelengths of 426.32 and 442.11 nm for PaL-AgNPs and PaR-AgNPs, respectively.

The FTIR absorption spectra showed clear and noticeable bands at 3030, 2121, 2013, 1938, 1745, 1575, 1284, and 643 cm^{-1} for PaL-AgNPs. These bands are similar to those seen at 3029, 2108, 1933, 1576, and 1222 cm^{-1} for PaR-AgNPs (Fig. 2). Additionally, the FTIR absorption spectra revealed closely related minor peaks at 3551, 2516, 1347, 1104, 1028, and 813 cm^{-1} for PaL-AgNPs, and 3536, 2509, 1741, 1361, 976, and 720 cm^{-1} for PaR-AgNPs. It is clear from these peaks that the AgNPs were made, capped, and kept stable by alkenes (=C–H–), esters (C=O), amides (N–H), and aliphatic amines (C–N).

The results from TEM analysis (Fig. 3A) clearly showed that the particles of both PaL-AgNPs and PaR-AgNPs were spherical in the presence of silver. While PaL-AgNPs

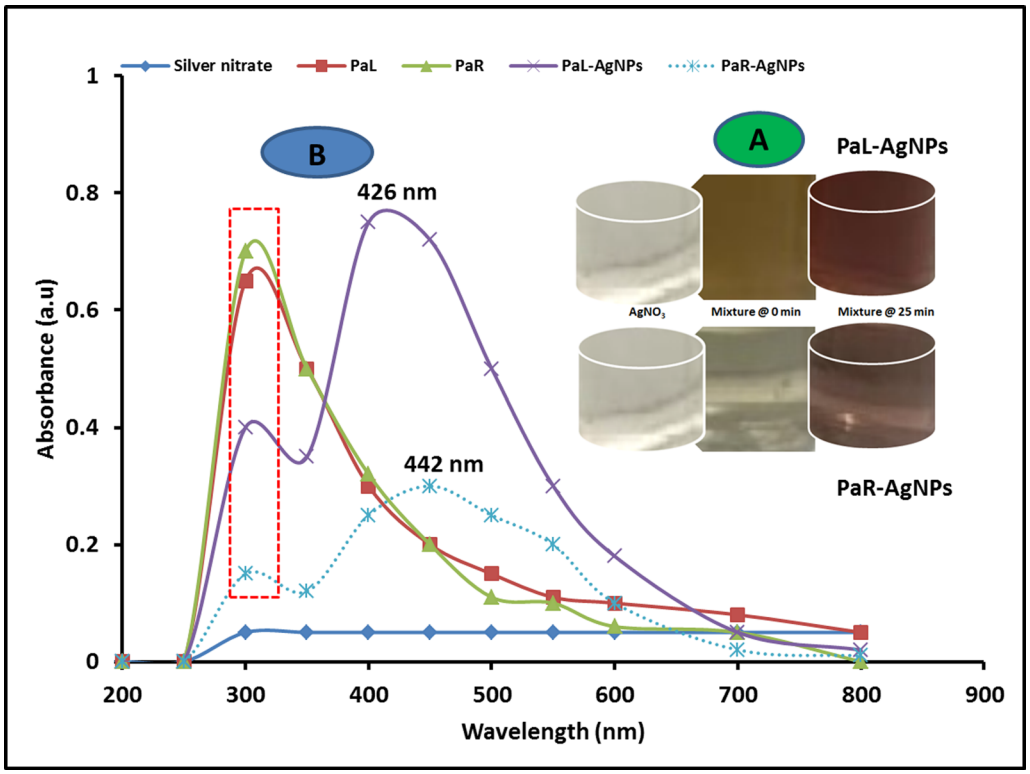


Fig. 1 The course of synthesis of AgNPs (A), and UV–Vis spectra of the nanoparticles, extracts and silver nitrate

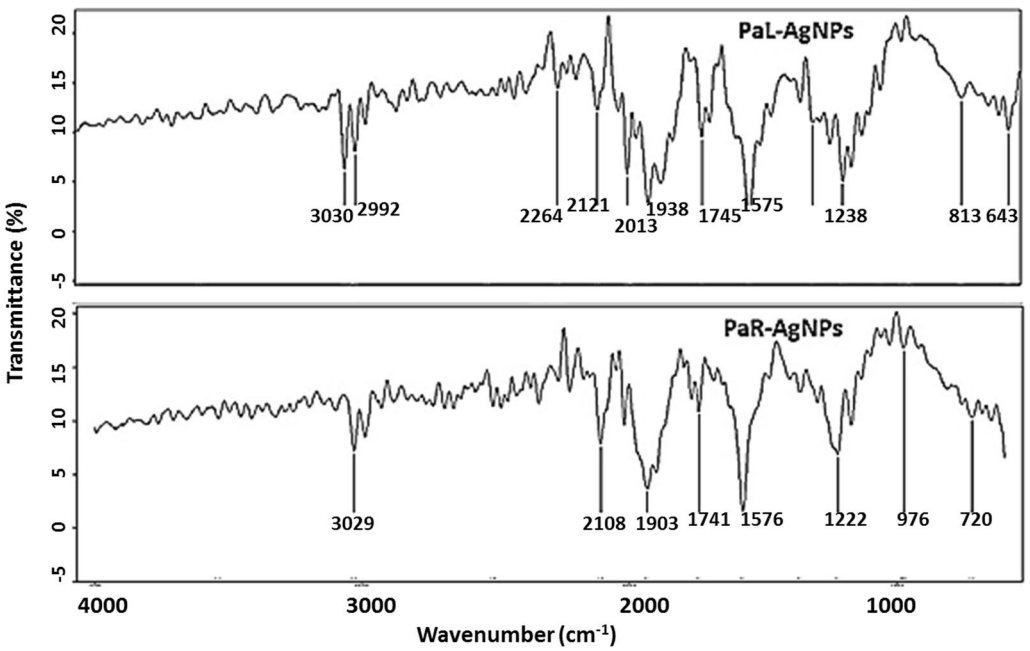


Fig. 2 FTIR spectra of Nano *P. alliacea* leaf (PaL-AgNPs) and Nano *P. alliacea* root (PaR-AgNPs)

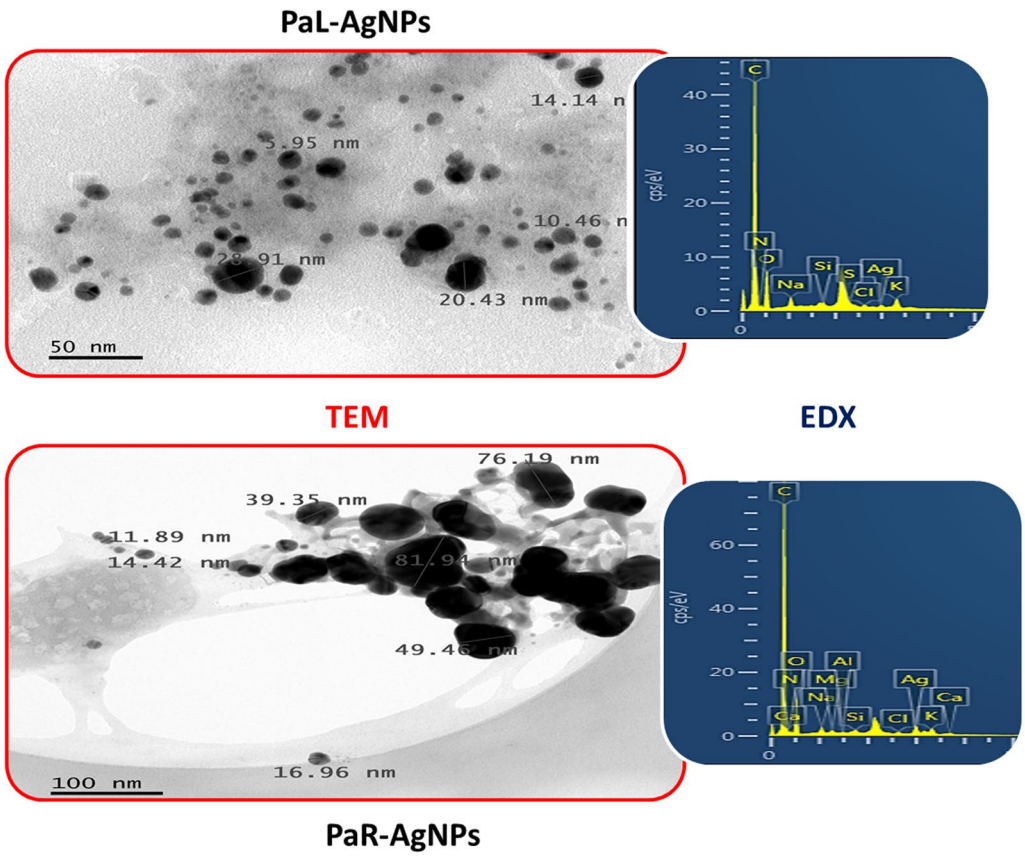


Fig. 3 The TEM micrographs (A) and EDX patterns (B) of the biosynthesized AgNPs

ranged in size from 5.95 to 28.91 nm, the PaR-AgNPs were 11.89 to 76.19 nm. EDX spectra (Fig. 3B) revealed the presence of Ag in the samples with *P. alliacea* leaf having 21.3% Ag while 16.3% Ag was observed in Pa-AgNPs. However, high incidence of carbon accounting for about 65–67% can be due to the organic moieties of the extracts.

3.2 Fumigant toxicity effects of insecticides on adult cockroaches

As presented in Table 1, the applied treatments exhibited significant control of adult cockroaches. After 1 h of exposure, lambdachyalothrin killed 100% of the cockroaches, while PaL-AgNPs at 10% v/v killed 33.3%, and PaR-AgNPs at 10% v/v killed 50% of the cockroaches. After two hours of exposure, 50.7% of the animals in the container treated with PaR-AgNPs and 66.7% of the animals in the container treated with PaL-AgNPs, both at a 10% concentration, died. There were no deaths in the containers treated with Pa-AgNPs, either at a 1% or 5% v/v concentration. However, after 3 h, applying 10% v/v of PaR-AgNPs and PaL-AgNPs killed all the tested insects. Using 5% v/v of PaR-AgNPs killed more insects (41.7, 66.7, and 91.7, respectively) at the same hours of exposure than using 5% v/v of PaL-AgNPs.

After 4 h of application, PaR-AgNPs at 1% caused a significantly higher mortality rate (25%) compared to PaL-AgNPs applied at the same rate. Cockroaches treated with PaL-AgNPs at 5% v/v experienced an increase in mortality after 16 h, while PaR-AgNPs and PaR-AgNPs at 5% v/v recorded 100% mortality at 16 h.

3.3 Contact toxicity effects of insecticides on adult cockroaches

Table 2 shows the effect of the contact toxicity of biosynthesised AgNPs on adult cockroaches. After 1 h of exposure, the application of PaR-AgNPs and PaL-AgNPs at 10% had the same significant effects on the cockroaches, while the cockroaches treated with PaR-AgNPs and PaL-AgNPs at 1% and 5% v/v showed no mortality. After 2 h of exposure, the lambdacyhalothrin-treated containers had 93.3% dead cockroaches, while the containers treated with PaR-AgNPs and PaL-AgNPs at 10% v/v had 50% mortality, and the container-treated PaL-AgNPs at 5% v/v had 8.3% dead cockroaches, while the cage treated with PaR-AgNPs

Table 1 Effects of insecticides on cockroaches (Fumigant Toxicity)

		Hours of exposure						
Treatments	**Rate**	**1**	**2**	**3**	**4**	**6**	**16**	**20**
Lambdacyhalothrin		100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}
Control		0.0^{d}	0.0^{c}	0.0^{c}	0.0^{d}	0.0^{d}	0.0^{c}	0.0^{c}
PaL-AgNPs	1	0.0^{d}	0.0^{c}	0.0^{c}	0.0^{d}	8.3^{cd}	16.7^{c}	33.3^{c}
	5	0.0^{d}	0.0^{c}	33.3^{b}	41.7^{c}	75.0^{b}	100.0^{a}	100.0^{a}
	10	33.3^{c}	66.7^{b}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}
PaR-AgNPs	1	0.0^{d}	0.0^{c}	8.3^{c}	25.0^{cd}	25.0^{c}	58.3^{b}	58.3^{b}
	5	0.0^{d}	0.0^{c}	41.7^{b}	66.7^{b}	91.7^{ab}	100.0^{a}	100.0^{a}
	10	50.0^{b}	50.0^{b}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}
Rates	1	25.0^{b}	27.1^{b}	27.1^{c}	31.3^{c}	35.4^{b}	41.7^{b}	48.0^{b}
	5	18.8^{c}	25.0^{b}	43.8^{b}	52.1^{b}	66.7^{a}	75.0^{a}	75.0^{a}
	10	45.8^{a}	54.2^{a}	75.0^{a}	75.0^{a}	75.0^{a}	75.0^{a}	75.0^{a}

Means with the same superscript(s) are significantly the same at 5% probability

Table 2 Effects of insecticides on cockroaches (Contact Toxicity)

		Hours of Exposure						
Treatments	**Rate**	**1**	**2**	**3**	**4**	**6**	**16**	**20**
Lambdacyhalothrin		80.0^{a}	93.3^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}
Control		0.0^{c}	0.0^{d}	0.0^{e}	0.0^{d}	0.0	0.0	0.0
PaL-AgNPs	1	0.0^{c}	0.0^{d}	0.0^{e}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}
	5	0.0^{c}	8.3^{c}	16.7^{d}	16.7^{c}	16.7^{c}	16.7^{c}	33.3^{c}
	10	25.0^{b}	50.0^{b}	75.0^{b}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}
PaR-AgNPs	1	0.0^{c}	0.0^{d}	0.0^{e}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}
	5	0.0^{c}	0.0^{d}	50.0^{c}	58.3^{b}	58.3^{b}	58.3^{b}	58.3^{b}
	10	25.0^{b}	50.0^{b}	75.0^{b}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}
Rates	1	32.5^{a}	48.3^{a}	62.5^{a}	75.0^{a}	75.0^{a}	75.0^{a}	75.0^{a}
	5	20.0^{b}	27.5^{b}	43.8^{b}	43.8^{b}	43.8^{b}	43.8^{b}	47.9^{b}
	10	20.0^{b}	23.3^{b}	25.0^{c}	25.0^{c}	25.0^{c}	25.0^{c}	25.0^{c}

Means with the same superscript(s) are significantly the same at 5% probability

at 5% showed no mortality. The lambdacyhalothrin-treated containers recorded 100% mortality, while the applied PaR-AgNPs and PaL-AgNPs at 10% killed 75% of cockroaches after 3 h, and the tested biosynthesized AgNPs at 10% achieved 100% mortality after 4 h of exposure. Throughout the experimental trials, none of the cockroaches treated with PaR-AgNPs and PaL-AgNPs at 1% v/v showed signs of death.

4 Discussion

The management of household insect pests using synthetic insecticides presents challenges, primarily due to their adverse environmental impacts and the significant costs associated with their acquisition. The application of nanoparticles for managing agricultural and household pests has gained significant support, attributed to their environmentally friendly characteristics and potent entomotoxic effects [31, 32]. In just 10 min, the root and leaf extracts of *P. alliacea* made it easier for the body to make nearly spherical AgNPs with a size range of 5.95–76.91 nm. Absorbance at these wavelengths is due to the unique surface plasmon resonance of the biosynthesized AgNPs, and the values here reported are within the range of 391–460 nm previously established in related existing literature for AgNPs [28, 30]. Lateef et al. [31] described how leaf aqueous extract from *P. alliacea* was used to make AgNPs and talked about their antimicrobial, anticoagulant, and antioxidant properties. The observed color changed of biosynthesized AgNPs goes in line with numerous reports that have documented the

brownish-colored biosynthesized AgNPs [27–29]. However, presence of phenolic compounds and the observed spherical shape in the synthesis of AgNPs are indications of biosynthesized AgNPs by the *P. alliacea* [31]. Currently, there is no documented evidence regarding the application of the plant's root extract to the synthesis of AgNPs. This report gives the first results of testing biogenic AgNPs made by *P. alliacea* to see how well they work as cockroach-killing insecticides. Therefore, we evaluated Pa-AgNPs on a common household insect (cockroach) using two application methods (contact and fumigant) at three different concentrations (1, 5, and 10% v/v).

The results showed that the biosynthesized AgNPs killed the tested insect, which strongly suggests that the tested Pa-AgNPs also killed insects. This observation is consistent with the findings of Yeguerman et al. [33], which highlighted the notable impacts of biosynthesized polymeric nanoparticles derived from peppermint, *Palmarosa geranium*, lavender, and rosemary on German cockroaches. Nonetheless, studies have reported on the extracts of *P. alliacea* utilized in the formulation of insecticides. For instance, studies have reported that *P. alliacea* oil can eliminate adult mosquitoes within 2 h of application [34]. In a similar vein, *P. alliacea* along with various other plant extracts were evaluated for their effectiveness against field insect pests affecting okra and cowpea [35, 36]. Nonetheless, current research indicates that the efficacy of *P. alliacea* in the formulation of nano-insecticides is linked to the organic compound known as dibenzyl trisulfide [37, 38]. The insecticidal effect of these Pa-AgNPs arises from the nanoparticles' capacity to easily bind and adsorb various chemical compounds, allowing for their circulation within insect body systems [39, 40]. Additionally, Azeez et al. [41] and Lateef et al. [30] showed that AgNPs had a lot of ability to get into cellular metabolism, which could change DNA and stop enzymes from working normally in the target organisms. Additionally, there have been reports indicating that nanoparticles impact the antioxidant levels and inhibit enzymes in insects, ultimately leading to their demise [42].

The efficacy of *P. alliacea* leaf and root in nano-insecticide formulations did not significantly differ, indicating that the formulation can utilize either part of the plant. Only 25% of the cockroaches studied died through contact within one hour of application, while exposure to the fumigant at the highest concentrations resulted in 50% mortality. Similarly, the fumigant's effects caused complete mortality within 3 h of exposure. In the meantime, a duration of 4 h was required to reach complete mortality via contact action. This implies that the introduction pathway of Pa-AgNPs plays a crucial role in controlling cockroach populations. However, the observed delayed insecticidal effects from contact toxicity may be because nano-insecticide formulations have a hard time penetrating cockroaches' cuticle. This indicates that Pa-AgNPs may exert a quicker lethal effect via the respiratory system. This observation supports the findings of Abdel-Raheem and Eldafrawy [43], who reported that silver nanoparticles applied as a fumigant were more effective in killing cockroaches than through contact toxicity. A previous study demonstrated that essential oil nanoparticles displayed insecticidal properties through direct contact with German cockroaches [44]. This indicates that the route of exposure is a factor that influences the toxicity of nano-insecticide formulations.

Regardless of the application method, the data collected indicated that the concentrations used influenced the effectiveness of the tested biosynthesized AgNPs. For example, a mortality rate of 75% in cockroaches was observed at a concentration of 10% v/v, whereas a mortality rate ranging from 43.8 to 52.1% was noted at 5% v/v. Simultaneously, a concentration of 1% eliminated 25–31.3% of the tested cockroaches after 3 h of application. This current investigation aligns with the previous findings by Abdel-Raheem and Eldafrawy [43], which indicated that the efficacy of silver nanoparticles against cockroaches is dependent on the dosage used.

While synthetic insecticide (lambdacyhalothrin) demonstrated a quicker lethality compared to biosynthesized AgNPs formulations, this suggests that the nano-insecticides exhibited delayed effects on the insect species studied, which is a prominent characteristic of botanical insecticides. However, the limitation of our study is the failure to investigate the mode of action of the insecticides on the tested insects. Nonetheless, biosynthesized nanoparticles are characterized as environmentally friendly owing to their reduced toxicity to both humans and the environment [31, 32]. Therefore, when considering the environmental benefits of nano-insecticides compared to their synthetic counterparts, we should not view the delayed effects of biosynthesized AgNPs on cockroaches as a definitive barrier to their application as an alternative to synthetic insecticides.

5 Conclusion

These findings demonstrated the effectiveness of *P. alliacea*-mediated silver nanoparticles in cockroach control. Also, either the leaf or root of *P. alliacea* can be used in the formulation of nano-insecticides. These nano-insecticide formulations act as contact and fumigant insecticides, though fumigant toxicity kills faster than contact toxicity. Application at the highest concentration, 10%, proved to be more effective than the other tested

concentrations. By using these AgNPs against household insects, we can safeguard our environment from harmful chemicals and lessen our reliance on synthetic insecticides. The formulation from *P. alliacea* can be a welcome development in the management of household pests, especially cockroaches. Therefore, the obtained result is significant as it paves the way for the development and commercialization of biosynthesized AgNPs using *P. alliacea* as industrial products in the near future.

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FAO, EJA, LA, ATA, and OOA conceptualized, curated the data, investigated, and design the experiment. G-KEB, BLS, and ME analyzed the data. All authors read and approved the manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

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Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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