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Enhancing the biological efficacy of clove extract via silver nano-loading to combat the stored drug beetle (Coleoptera: Anobiidae)

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تعزيز الفعالية الحيوية لمستخلص القرنفل عبر التحميل النانوي للفضة لمكافحة خنفساء العقاقير المخزونة (رتبة غمدية الأجنحة: فصيلة أنوبيدي)

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الملخص:
أدت المخاوف البيئية المتزايدة وتنامي مقاومة الحشرات للمبيدات إلى البحث عن مبيدات حشرية نباتية مستدامة لتحل محل المبيدات الاصطناعية في حماية المنتجات المخزنة. ولتلبية هذه الحاجة، قيّمت هذه الدراسة سمية مستخلصات القرنفل عن طريق التلامس، باستخدام كلٍ من المستخلصات المائية والإيثانولية، بالإضافة إلى مستحضر جديد من المستخلص المائي المُعزز بجزيئات الفضة النانوية ، ضد خنفساء العقاقير المخزونة ،. أُجريت اختبارات حيوية على ورق الترشيح بتراكيز 5٪ و10٪ و15٪ و20٪ (حجم/حجم)، وسُجلت نسبة النفوق بعد 6 و12 و24 ساعة. أظهرت النتائج أن التوصيف البنيوي (المجهر الإلكتروني الماسح، مطيافية تشتت طاقة الأشعة السينية، مطيافية الأشعة تحت الحمراء بتحويل فورييه) أكد نجاح تصنيع جزيئات الفضة النانوية الكروية البلورية بمتوسط قطر 28.34 ± 1.203 نانومتر، والتي تم تثبيتها بواسطة مكونات نباتية متعددة الفينولات. كان المستخلص المُعزز بجزيئات الفضة النانوية هو التركيبة الأقوى، حيث بلغ التركيز النصف المميت (LC_{50}) بعد 24 ساعة 1.52 ٪. ويمثل هذا زيادة في الفعالية بمقدار 4.34 ضعفًا مقارنةً بالمستخلص المائي وحده 6.60 ٪، بينما بلغ التركيز النصف المميت في المستخلص الإيثانولي 2.50٪. وأكد التحليل الإحصائي (ANCOVA) أن تعزيز الجسيمات النانوية أدى إلى زيادة ملحوظة في معدل النفوق بنسبة 21٪ تقريبًا. تُظهر هذه النتائج أن المستخلصات القائمة على القرنفل، وخاصةً التركيبة النانوية، تُشكل بديلاً واعدًا وقابلاً للتحلل الحيوي للمبيدات الحشرية الاصطناعية لحماية المنتجات المخزنة.

الكلمات الدالة: مبيد حشري نباتي، مستخلص القرنفل، ستيجوبيوم بانيسيوم، سيزيجيوم أروماتيكوم، جزيئات نانوية من الفضة.

Abstract
Growing environmental and resistance concerns have spurred the search for sustainable botanical insecticides to replace synthetic fumigants in stored-product protection. To address

this need, this study evaluated the contact toxicity of clove (*Syzygium aromaticum*) extracts using both aqueous and ethanolic extracts as well as a novel formulation of silver nanoparticle-enhanced aqueous extract (AgNPs) against adults of the drugstore beetle, *Stegobium paniceum*. Filter paper bioassays were conducted at concentrations of 5%, 10%, 15%, and 20% (v/v), with mortality recorded after 6, 12, and 24 hours. Results demonstrated that the structural characterization (SEM, EDX, FT-IR) confirmed the successful synthesis of spherical, crystalline AgNPs with an average diameter of 28.34 ± 1.203 nm, stabilized by polyphenolic phytoconstituents. The AgNP-enhanced extract was the most potent formulation, exhibiting a 24-hour LC_{50} of 1.52 % . This represents a 4.34-fold increase in potency over the aqueous extract alone (LC_{50} = 6.60%), while the ethanolic extract had an LC_{50} of 2.50%. Statistical analysis (ANCOVA) confirmed that the nanoparticle enhancement caused a significant increase in mortality of approximately 21%. These results demonstrate that clove-based extracts, particularly the nano-formulation, present a promising and biodegradable alternative to synthetic insecticides for protecting stored products.

Keywords: botanical insecticide, clove extract, Stegobium paniceum, Syzygium aromaticum, , silver nanoparticles**.**

Introduction

Stegobium paniceum L. (Coleoptera: Anobiidae), commonly known as the drugstore beetle, is a cosmopolitan pest that infests a wide range of dried stored products, including pharmaceuticals, spices, grains, and dried herbs (**Papadopoulou and Buchelos, 2002**). Its capacity to penetrate packaging materials, combined with its high reproductive rate, results in substantial economic losses and contamination (**Rees, 2004**). While a variety of fumigants and synthetic pesticides are commonly used for control, their overuse has led to serious challenges, notably the development of insect resistance. Pesticide resistance is a growing concern in stored cereal preservation globally. In several countries, resistance has been documented in key stored-product insect pests (**Talukder, 2009**). For instance, as reported by **DARP (2003)**, 122 insect pest species have developed resistance to malathion. There are also increasing reports of widespread phosphine resistance in various stored grain insects across multiple countries, which in some cases has led to control failures (**Chaudhry, 1997**). These issues have intensified the search for alternative formulations to replace conventional chemical treatments.

Botanical insecticides, which are derived from plant secondary metabolites, present a promising pest management strategy. They are favored for their low mammalian toxicity, rapid environmental degradation, and multiple, novel modes of action that complicate pest resistance (**Isman, 2020; Demirak and Canpolat, 2022**). As biocompounds sourced from various plant species, they offer a more sustainable and eco-friendly alternative to conventional pesticides **(Akbar et al., 2024**).

Clove (*Syzygium aromaticum*) is the dried aromatic flower bud of an evergreen tree in the Myrtaceae family, used both as a spice and in herbal medicine (**Singh et al., 2015**). Clove buds and their essential oil are known for their broad biological activities, including antibacterial, antioxidant, antiparasitic, antimutagenic, antifungal, and antithrombic effects (**Kumar et al., 2014; Giordani et al., 2004**). The clove's bioactivity is largely attributed to its high concentration of phenolic compounds, primarily eugenol and eugenyl acetate (**Hashem et al., 2020; Ikawati et al., 2021; Charfi et al., 2021; Parichanon et al., 2025**). For instance, eugenol constituted 74% of an ethanolic clove extract and 43% of an aqueous extract (**Dibazar et al., 2015**), findings consistent with earlier reports by **Chaieb et al. (2007)** and **Lee et al. (2009).** Other major constituents include β-caryophyllene and eugenyl acetate.

S. aromaticum is strategically important across multiple industries, such as pharmaceuticals, cosmetics, and food and beverages (**Cortés-Rojas et al., 2014**). Recently, there has been growing interest in its potential for pest control, with studies reporting insecticidal and repellent effects against stored-product pests like *Sitophilus zeamais* (**Aryal et al., 2023**), *Rhyzopertha dominica* (**Zeng et al., 2010**), *S. oryzae* (**Hamed et al., 2023; Bandara and Senevirathne, 2023**), and *Tribolium castaneum* (**Mishra et al., 2016; Elnabawy et al., 2021**). However, the scientific literature on this application remains limited,

and comprehensive toxicological studies on clove extracts for insects are still needed (**Pandey and Singh, 2011**).

Recent advancements in bio-nanotechnology have opened new frontiers in pest management. In particular, the plant-mediated synthesis of silver nanoparticles (AgNPs) has attracted significant interest due to its enhanced antimicrobial and insecticidal properties (**Rouhani et al., 2012**). This approach can create a synergistic effect, improving the stability, bioavailability, and targeted toxicity of botanical compounds (**Murugan et al., 2015**). For instance, AgNPs fabricated with duck weed (*Lemna minor*) extract demonstrated high contact toxicity against *S. oryzae* (**Abdel-Megeed et al., 2024**). Similarly, studies have shown remarkable efficacy: clove oil-based AgNPs achieved 71% repellency and 100% larvicidal mortality against *Tribolium castaneum* (**Selvaraj et al., 2019**), while those synthesized from *Moringa oleifera* leaf extract caused 100% mortality in *S. oryzae* (**Rani et al., 2019**). Furthermore, AgNPs have proven lethal against *Oryzaephilus surinamensis*, achieving complete mortality at 5,000 ppm within 72 hours (**Salman and Hameed, 2020**). The present study employs an aqueous extract of clove buds for the green synthesis of silver nanoparticles (AgNPs), thereby avoiding hazardous chemical stabilizers and reducing agents. Then characterize and compare the contact toxicity of these AgNPs, prepared with aqueous extract, with that of aqueous and alcoholic clove extracts against adult drugstore beetles. The findings are expected to provide technical support for developing plant-based insecticidal strategies and for enhancing the efficacy of extracts for protection, offering promising alternatives to traditional synthetic pesticides.

Materials and Methods

2.1. Plant material

Clove (*Syzygium aromaticum* L., family Myrtaceae) used in this study were collected from the local market and had their identity confirmed by a botanist.. Subsequently, the cloves were powdered using a pestle and mortar.

2.2. Insect Rearing

A laboratory colony of *S. paniceum* was maintained on a diet of whole wheat flour and yeast (10:1 w/w) at 28 ± 2°C, 65 ± 5% RH, and a 12:12 h (L:D) photoperiod. Unsexed adults (1-7 days old) were used for all bioassays.

2.3. Preparation of extracts and nanoformulation

2.3.1. Preparation of aqueous extract of clove

A clove buds extract was prepared by dissolving 50 g of powdered clove in 250 mL of distilled water within a conical flask. After covering the flask with cotton wool and foil paper, the mixture was agitated thoroughly. It was then allowed to macerate for 24 hours with regular shaking. Following maceration, the mixture was filtered through Whatman no. 1 filter paper. The collected filtrate was centrifuged, and the supernatant was transferred into a sterile Falcon tube after filtration and stored at 4°C in a refrigerator for future use.

2.3.2. Preparation of ethanolic extract of clove

Fifty grams of powdered clove buds were combined with 250 mL of 95% ethanol in a conical flask. The flask was sealed with foil and cotton wool, and then shaken thoroughly. The mixture was allowed to stand for 24 hours before being filtered through Whatman no. 1 filter paper. The resulting filtrate was centrifuged at 2000 rpm for 5 minutes. The supernatant was decanted, filtered again, and concentrated using a rotary evaporator. The final extract was stored in a sterile falcon tube at 4°C until further use.

2.3.3. Preparation of clove aqueous extract with Silver Nanoparticles

Silver nanoparticles (AgNPs) were synthesized following the biogenic reduction method described by **Abdel-Megeed et al. (2024)**. Briefly, 90 mL of 1 mM silver nitrate ($AgNO_3$) was combined with 10 mL of crude clove extract in a flask. The mixture was heated at 60 °C for 45 minutes under continuous magnetic stirring. To prevent photo-degradation of the light-sensitive silver ions, the flask was wrapped in aluminum foil. After heating, the reaction solution was kept in complete darkness for 24 hours to ensure thorough reduction. Successful formation of AgNPs was indicated by a color change from pale brown to dark brown. For characterization the resulting suspension was centrifuged at 12,000 rpm for 20 minutes to collect the AgNPs. The supernatant was discarded, and the pellet was washed three times

with distilled water. The washed nanoparticles were dried at 80 °C, gently ground with a few drops of ethanol to obtain a fine powder, and stored at 7 °C for further characterization.

2.4 Characterization of the biosynthesized AgNPs

The elemental composition of the synthesized nanoparticles was determined using Energy-Dispersive X-ray Spectroscopy (EDX) coupled with a scanning electron microscope (SEM, JSM-IT200, JEOL, Japan). The analysis was performed at an acceleration voltage of 20 kV, a working distance of 10 mm, and a magnification of ×170. Spectra were acquired under high vacuum with a live time of 30 seconds. Quantitative analysis was conducted using the standardless ZAF correction method.

Fourier-transform infrared (FTIR) spectroscopy was conducted using a Perkin-Elmer SPECTRUM 10 spectrometer (USA) based on the technique described by **Faisal et al. (2020).** Liquid samples were combined with KBr to form solid disks for analysis. Spectra were recorded in the range of 450–4000 cm^{-1} at a resolution of 8 cm^{-1}. The resulting spectrum was interpreted by assigning vibrational bands to specific functional groups in order to identify capping and reducing agents present on the surface of the AgNPs.

2.5. Bioassay

For the contact bioassay, 1 mL of each test concentration (5, 10, 15, and 20%) was applied uniformly to Whatman No.1 filter papers (9 cm diameter) inside petri dishes (9 x 1.6 cm). Control papers were treated with 1 mL of distilled water (aqueous extracts) or 95% ethanol (ethanolic extracts). Once the solvent evaporated, five adult beetles were placed in each dish, with three replicates per treatment. Beetle mortality was assessed at 6, 12, and 24 hours.

2.6. Statistical Analysis

The particle size distribution was modeled using a log-normal probability density function of the form:

$$f(d)=\frac{1}{d\sigma\sqrt{2\pi}}\exp\left(-\frac{(\ln d-\mu)2}{2\sigma 2}\right)$$

where d is the particle diameter, μ is the mean of the natural logarithm of the diameter, and σ is the standard deviation of the log-transformed data.

Concentration-mortality data were analyzed using a two-pronged statistical approach. First, probit regression was employed to estimate lethal concentration values (LC_{50}, LC_{90}) with 95% fiducial confidence intervals, with log10-transformed concentrations. Goodness-of-fit was assessed using Pearson's chi-square test, with $p > 0.05$ indicating adequate model fit. Parallelism of dose-response curves among extract types was tested, with non-significant results ($p > 0.05$) indicating similar slopes and validating LC_{50} comparisons. Relative median potency ratios with 95% confidence intervals were calculated to compare extract efficacy. For integrated efficacy assessment, two-way ANCOVA was performed on arcsine-square-root transformed mortality proportions, with extract formulation and exposure time as fixed factors and concentration as a covariate. Model assumptions were validated (Levene's test: $F_{8,27} = 1.23$, $p = 0.312$; Shapiro-Wilk: $W = 0.974$, $p = 0.476$). Bonferroni-adjusted pairwise comparisons were conducted for significant effects. Effect sizes are reported as partial eta-squared (ηp^2) with 95% CIs. Analyses were performed using SPSS Statistics (Version 28, IBM Corp.) with $\alpha = 0.05$.

Results

3.1. Structural Characterization of Ag-NPs

3.1.1 Surface morphology and droplet size distribution

The surface morphology, particle size, and crystalline nature of the synthesized silver nanoparticles (AgNPs) were analyzed using scanning electron microscopy (SEM). Representative SEM images (Figure 1) show that the nanoparticles are predominantly spherical and uniformly dispersed, with minimal aggregation. The observed separation between particles suggests successful stabilization by the capping agent. Analysis of multiple images determined an average particle size of 28.34 nm, with a standard deviation of 1.203 nm. To statistically quantify the size distribution, a histogram of particle sizes was fitted to a log-normal distribution function, as shown in Figure 2.

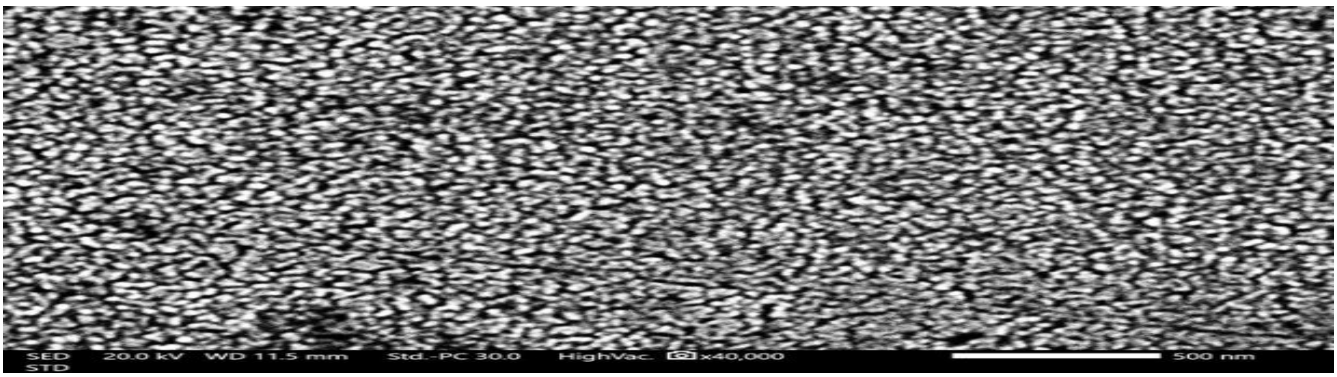

Fig. 1. Scanning electron micrograph showing the spherical morphology of the prepared formulations under the following imaging conditions: 20.0 kV, high vacuum, magnification 40,000x. Scale bar: 500 nm.

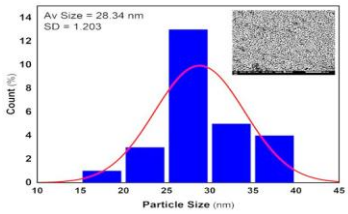

Fig. 2. Size distribution histogram of synthesized silver nanoparticles (AgNPs) fitted with a log-normal distribution function with the average (Av) size and standard deviation (SD) derived from the fitted curve is 28.34±1.203 nmFigure 1: Type here (10, Times New Roman).

3.1.2 Energy-dispersive X-ray (EDX) spectroscopic analysis of silver nanoparticles

The elemental composition of the synthesized Ag-NPs, energy-dispersive X-ray (EDX) spectroscopy coupled with scanning electron microscopy (SEM) was employed. The EDX spectrum exhibited a prominent peak for silver (Ag), with a mass percentage of 43.87 ± 0.62% and an atomic percentage of 10.53 ± 0.15%, confirming the successful formation of silver nanoparticles. Additional peaks corresponding to carbon (C, 44.34 ± 0.68%) and oxygen (O, 34.04 ± 1.06 %) were also observed, likely originating from the extracellular components of clove bud extract capping agents or surface adsorbates present in the system. Trace amounts of sodium (Na), magnesium (Mg), silicon (Si), sulfur (S), potassium (K), and calcium (Ca) were detected, which may be attributed to phytoconstituents derived from the clove bud extract used in the synthesis.

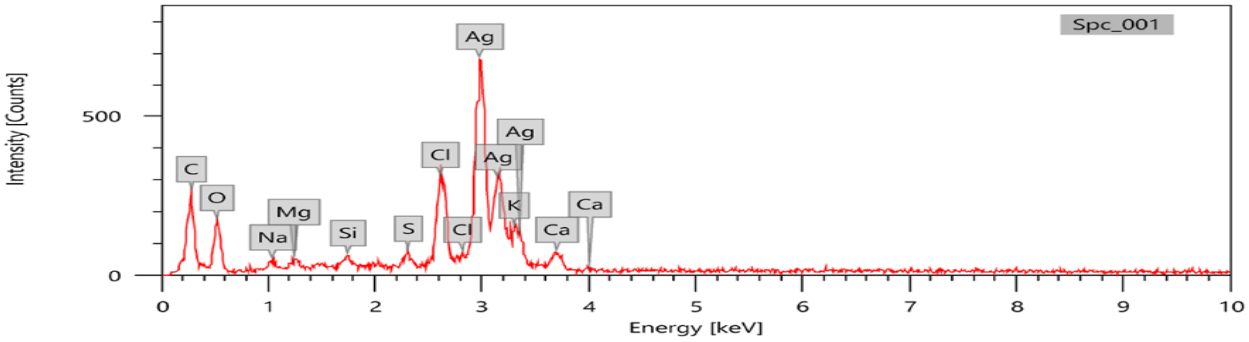

Fig. 3. Energy-dispersive X-ray (EDX) spectrum of the synthesized nanoparticles. The inset table lists the quantitative elemental composition (weight and atomic percentages) derived from the spectrum, confirming the presence of silver as the primary metallic component. The prominent carbon and oxygen signals are attributed to the extracellular components of clove bud extract capping agents used during synthesis.

3.1.3 Fourier transform infrared spectroscopy (FT-IR)

Fourier-transform infrared spectroscopy was used to characterize the functional groups in the clove extract responsible for the reduction of Ag^+ ions and stabilization of the resulting nanoparticles. The FTIR spectrum (Figure 4) displayed several distinct absorption bands indicative of bioactive phytochemicals.The most prominent feature was a broad, intense band centered at 1636.2 cm^{-1}, characteristic of the stretching vibrations of carbonyl groups (C=O) in conjugated ketones, amides, or quinones. This band is frequently associated with flavonoid and terpenoid constituents, which are known to participate in redox reactions. Accompanying this were signals in the hydroxyl region: a broad band at 3356.9 cm^{-1} and a shoulder at 3256.2 cm^{-1}, corresponding to O–H stretching vibrations of phenolic compounds and alcohols. These groups are critical for the chelation and reduction of metal ions.

Further evidence for polyphenolic structures was provided by peaks at 1516.4 cm^{-1} and 1457.0 cm^{-1}, assignable to aromatic C=C ring stretching and C–H bending modes, respectively. The region between 1272–1349 cm^{-1}, with peaks at 1349.6 cm^{-1}, 1272.4 cm^{-1}, and 1251.6 cm^{-1}, is consistent with C–O stretching and O–H deformation vibrations of phenolic acids. Additionally, the strong peak at 1084.6 cm^{-1} suggests the presence of C–O–C ether linkages or C–O stretching in polysaccharides or glycosidic bonds, while the band at 946.0 cm^{-1} may arise from C–C bending or =C–H bending vibrations. The quantitative peak reveals that the bands at 1636.2 cm^{-1} and 1084.6 cm^{-1} possessed the largest negative areas (-1494.6 and -586.2, respectively), indicating particularly strong absorption. This suggests that the functional groups corresponding to these wavenumbers namely, carbonyls and ethers/polysaccharides are present in high concentration and are likely major participants in the bioreduction and capping processes.

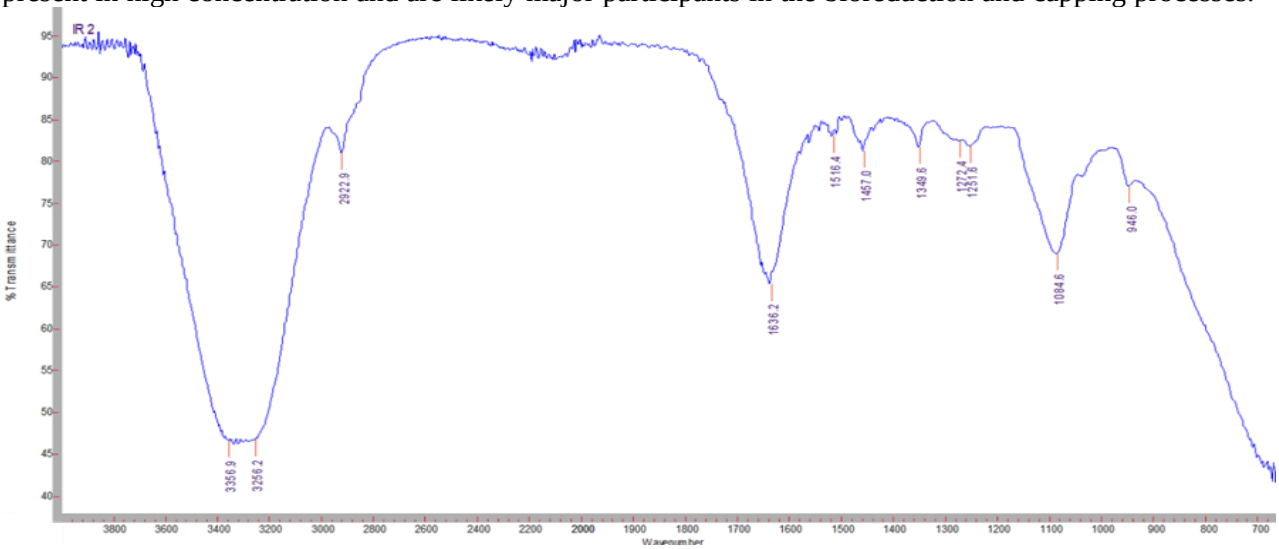

Fig. 4. Fourier-transform infrared (FTIR) spectrum of clove extract. Key absorption bands are labeled with their corresponding wavenumbers (cm^{-1}) and assigned functional groups. The broad bands in the 3200–3400 cm^{-1} region (O–H stretch) and the intense peak at ~1636 cm^{-1} (C=O stretch) indicate the presence of polyphenolic and carbonyl-containing compounds, which are implicated in the reduction of Ag^+ ions and stabilization of the resulting nanoparticles.

3.2. Probit Analysis of Lethal Concentrations (LC_{50} and LC_{90})

Probit analysis revealed concentration- and time-dependent mortality for all three clove extract formulations against *S. paniceum* adults. The lethal concentrations required to kill 50% (LC_{50}) and 90% (LC_{90}) of the test population decreased with increased exposure time for each extract type, indicating a cumulative toxic effect (Table 1). The aqueous extract was the least potent formulation across all time points. Its 24-hour LC_{50} was 6.60 ppm (95% CI: 1.83-12.70). Both the alcoholic extract and the aqueous extract enhanced with silver nanoparticles (AgNPs) demonstrated significantly higher potency. The

alcoholic extract was consistently more toxic than the aqueous extract. At 24 hours, its LC_{50} was 2.50 ppm (95% CI: 0.70-5.74), which was 2.64 times more potent than the aqueous standard, although this relative potency was not statistically significant (95% CI: 0.48-9.20). The most effective formulation was the aqueous extract combined with AgNPs. It exhibited the lowest LC_{50} values at each time point, culminating in a 24-hour LC_{50} of 1.52 ppm (95% CI: 0.56-4.17). This represents a statistically significant 4.34-fold increase in potency compared to the aqueous extract alone at 24 hours (95% CI: 1.59-9.09). A significant potency increase for the alcoholic extract was also observed at the 6-hour exposure (3.55-fold, 95% CI: 1.05-16.67). The model fit and parallelism demonstrated a good fit to the observed mortality data for all analyses, as indicated by non-significant chi-square (χ^2) values ($p > 0.05$) (Table 1). Furthermore, tests for parallelism confirmed that the dose-response regression lines for the different extract types were parallel at each respective time point ($p > 0.05$), validating the comparisons of relative potency.

Table 1. Probit analysis of lethal concentrations (LC_{50} and LC_{90}) for clove extract formulations against *S. paniceum* adults over time.

Extract Type	Time (hour)	LC_{50} (95% CI)	LC_{90} (95% CI)	Slope	Intercept	χ^2 (Fit)	p (Parallel)	Relative Potency (95% CI)
Aqueous	6	16.82 (6.90-29.80)	58.33 (47.50-77.00)	0.946	-1.160	0.241	0.946	1
	12	9.26 (2.41-21.60)	19.11 (14.62-29.60)	1.099	-1.062	0.386	0.959	1
	24	6.60 (1.83-12.70)	11.01 (3.55-20.60)	1.162	-0.952	0.639	0.983	1
Alcoholic	6	4.74 (0.90-10.50)	13.98 (4.39-28.40)	0.946	-1.800	0.241	0.946	3.55 (1.05-16.67)*
	12	3.58 (1.78-7.59)	7.10 (1.83-15.90)	1.099	-1.671	0.386	0.959	2.59 (0.49-8.10)
	24	2.50 (0.70-5.74)	3.92 (1.34-10.60)	1.162	-1.414	0.639	0.983	2.64 (0.48-9.20)
Aqueous + Silver NPs	6	5.29 (1.02-10.50)	15.49 (4.90-25.40)	0.946	-1.844	0.241	0.946	3.18 (0.94-8.12)
	12	3.95 (0.92-8.20)	7.80 (2.02-20.00)	1.099	-1.718	0.386	0.959	2.34 (0.53-10.30)
	24	1.52 (0.56-4.17)	2.94 (1.05-7.82)	1.162	-1.163	0.639	0.983	4.34 (1.59-9.09)*

LC values are expressed as percentage concentration (w/v). 95% confidence intervals are in parentheses. Relative potency is calculated in comparison to the aqueous extract at the corresponding exposure time.. χ^2 (Fit) = Pearson goodness-of-fit chi-square (all $p = 1.000$, indicating excellent model fit). p (Parallel) = Parallelism test p-value (all >0.94, indicating parallel slopes). *Statistically significant (95% CI does not include 1.00).

3.3. Insecticidal Activity of Clove Extract Formulations

The insecticidal efficacy of three extract formulations, aqueous, ethanolic, and silver nanoparticle-enhanced aqueous extract (AgNPs) as shown in Table 2, was evaluated at concentrations of 5%, 10%, 15%, and 20% over exposure periods of 6, 12, and 24 hours, using three biological replicates of five insects each. Mortality increased with both concentration and exposure time across all treatments. The silver nanoparticle-enhanced aqueous extract (AgNPs) consistently demonstrated superior efficacy, achieving the highest mortality at all time points. At 20% after 24 hours, mortality was 93.3% ± 25.2, representing a 27.3% improvement over ethanolic extract (86.7% ± 15.3) and a 56.0% improvement over clove aqueous extract (73.3% ± 17.6) at the same concentration and duration. The silver nanoparticle-enhanced aqueous extract (AgNPs) produced synergistic enhancement, increasing average mortality by 38.8% compared to aqueous extract alone across all concentrations.

Table2. Effect of Clove alcoholic and aqueous and silver nanoparticle-enhanced aqueous extract on the mortality of *S. paniceum* adults over time.

Plant	**Extract Type**	**Conce** (%)	**Mortality** (% ± SD)		
			6h	12h	24h
Clove	**Aqueous**	5	33.3 ± 13.3	40.0 ± 13.3	46.7 ± 17.6
		10	40.0 ± 13.3	46.7 ± 17.6	53.3 ± 20.8
		15	46.7 ± 17.6	60.0 ± 13.3	66.7 ± 23.1
		20	53.3 ± 20.8	66.7 ± 23.1	73.3 ± 15.3
	Alcoholic	5	53.3 ± 20.8	60.0 ± 13.3	66.7 ± 23.1
		10	60.0 ± 13.3	66.7 ± 23.1	73.3 ± 15.3
		15	66.7 ± 23.1	73.3 ± 15.3	80.0 ± 10.0
		20	73.3 ± 15.3	80.0 ± 10.0	86.7 ± 5.8
	silver nanoparticle-enhanced aqueous extract	5	46.7 ± 17.6	53.3 ± 20.8	73.3 ± 15.3
		10	60.0 ± 13.3	66.7 ± 23.1	80.0 ± 10.0
		15	66.7 ± 23.1	73.3 ± 15.3	86.7 ± 5.8
		20	73.3 ± 15.3	80.0 ± 10.0	93.3 ± 25.2
Control		0	00.0±00.0	00.0±00.0	00.0±00.0

Two-way ANCOVA (Table 3), revealed statistically significant main effects of extract formulation ($F_{2, 27} = 27.16$, $p < 0.001$, $\eta^2_p = 0.668$, 95% CI: 0.434−0.791), exposure time ($F_{2, 27} = 32.90$, $p < 0.001$, $\eta^2_p = 0.709$, 95% CI: 0.508−0.828), and concentration ($F_{1, 27} = 83.61$, $p < 0.001$, $\eta^2_p = 0.756$, 95% CI: 0.583−0.854) on insect mortality. The extract × time interaction was not statistically significant ($F_{4, 27} = 0.94$, $p = 0.456$, $\eta^2_p = 0.122$, 95% CI: 0.000−0.336) and was therefore excluded from the final model. The fitted ANCOVA model explained 92.5% of the variance in mortality ($R^2 = 0.925$, adjusted $R^2 = 0.902$), with concentration alone accounting for 75.6% of the explained variance.

Comparative efficacy of clove extract formulations, Bonferroni-adjusted pairwise comparisons indicated substantial differences among extract formulations (Table 4). Aqueous extract produced significantly lower mortality than both alcoholic extract (mean difference = -0.189, 95% CI: -0.263 to -0.115, $t_{27} = -5.11$, $p < 0.001$, Bonferroni-adjusted $p < 0.001$) and nanoparticle-enhanced extract (mean difference = -0.208, 95% CI: -0.282 to -0.134, $t_{27} = -5.62$, $p < 0.001$, Bonferroni-adjusted $p < 0.001$) after controlling for concentration and exposure time. No statistically significant difference was detected between alcoholic and nanoparticle-enhanced extracts (mean difference = -0.019, 95% CI: -0.093 to 0.055, $t_{27} = -0.51$, $p = 0.612$, Bonferroni-adjusted $p = 0.870$).

The parameter estimates from the ANCOVA model (Table 3), indicate that, when holding concentration and exposure time constant, alcoholic extract increased mortality by 0.189 units on the

arcsine scale (equivalent to approximately 18.9% increase in raw mortality probability) compared to aqueous extract, while nanoparticle enhancement provided a similar improvement of 0.208 units (20.8% increase).

Exposure duration significantly influenced mortality, with progressive increases observed from 6 to 24 hours (Table 3). Compared to 24-hour exposures, 6-hour exposures resulted in significantly lower mortality (mean difference = -0.098, 95% CI: -0.161 to -0.035, $t_{27} = -2.65$, $p = 0.013$, Bonferroni-adjusted $p = 0.053$), p=0.053), while 12-hour exposures showed intermediate effects that did not differ significantly from either 6-hour (mean difference = 0.083, 95% CI: -0.020 to 0.186, $t_{27} = 2.24$, $p = 0.033$, Bonferroni-adjusted $p = 0.130$) or 24-hour exposures (mean difference = -0.015, 95% CI: -0.078 to 0.048, $t_{27} = -0.41$, $p = 0.687$, Bonferroni-adjusted $p = 0.625$).

Concentration exhibited a strong positive linear relationship with mortality ($\beta = 0.024$, 95% CI: 0.019−0.030, $t_{27} = 9.14$, $p < 0.001$). Each 1% increase in concentration was associated with a 0.024-unit increase in arcsine-transformed mortality, corresponding to approximately a 2.4% increase in raw mortality probability across the tested concentration range (5-20%).

Table 3. Analysis of covariance (ANCOVA) for insecticidal activity

Source	SS	df	MS	F	p	Partial η^2	95% CI for η^2
Corrected Model	2.917	8	0.365	41.43	<0.001	0.925	0.834-0.962
Intercept	0.015	1	0.015	1.73	0.198	0.060	0.000-0.272
Concentration	0.737	1	0.737	83.61	<0.001	0.756	0.583-0.854
Extract Type	0.479	2	0.239	27.16	<0.001	0.668	0.434-0.791
Exposure Time	0.580	2	0.290	32.90	<0.001	0.709	0.508-0.828
Extract × Time	0.033	4	0.008	0.94	0.456	0.122	0.000-0.336
Error	0.238	27	0.009				
Total	34.241	36					
Corrected Total	3.155	35					

Dependent variable = arcsine-square-root transformed mortality proportion. Model $R^2 = 0.925$, Adjusted $R^2 = 0.902$. Concentration was treated as a continuous covariate. SS = Sum of Squares, MS = Mean Square.*

Table 4. Pairwise comparisons with Bonferroni adjustment

Comparison	Parameter	M D	SE	t	p	Bonferroni p	95% CI
Extract Type	Aqueous vs. Alcoholic	-0.189	0.037	-5.11	<0.001	<0.001	-0.263 to -0.115
	Aqueous vs. Nano-enhanced	-0.208	0.037	-5.62	<0.001	<0.001	-0.282 to -0.134
	Alcoholic vs. Nano-enhanced	-0.019	0.037	-0.51	0.612	0.870	-0.093 to 0.055
Exposure Time	6h vs. 12h	0.083	0.037	2.24	0.033	0.130	-0.020 to 0.186
	6h vs. 24h	-0.098	0.037	-2.65	0.013	0.053	-0.161 to -0.035
	12h vs. 24h	-0.015	0.037	-0.41	0.687	0.625	-0.078 to 0.048

M D = Mean differences are on arcsine-transformed scale. Negative values indicate lower mortality for first group in comparison. SE = Standard Error, CI = Confidence Interval.

Discussion

This study successfully demonstrates the synthesis of silver nanoparticles (AgNPs) using clove bud (*Syzygium aromaticum*) extract and establishes the superior insecticidal efficacy of the nanoparticle-enhanced formulation against *S. paniceum* adults. The findings align with **Bansal et al. (2018), Abdel-Megeed et al. (2024), and Shukla et al. (2025)** that the growing body of literature advocating for green-synthesized nanoparticles as potent, eco-friendly alternatives to conventional pesticides also provides nuanced insights into structure-activity relationships.

Our finding confirms the successful bioreduction of Ag^+ ions and the formation of stable, nanoscale silver. Where the SEM analysis revealed predominantly spherical nanoparticles with an average size of 28.34 nm and minimal aggregation, this finding is consistent with the findings of **Abdul-Zahra et al. (2025)**, who revealed that the manufactured Ag-*Triticum aestivum* L nanoparticles had a diameter of 52.14-96.16 nm. Furthermore, **Abdel-Megeed et al. (2024)** observed that SEM micrographs revealed spherical-shaped generated AgNPs with an average size of 40.56 nm. This uniform morphology and dispersion are critical, as particle size and shape directly influence biological activity, with smaller particles typically exhibiting greater reactivity and cellular penetration (**Rai et al., 2018; Ranjani et al., 2020; Deka et al., 2021**).The log-normal size distribution is typical for green-synthesized nanoparticles and indicates a controlled nucleation and growth process mediated by the phytochemicals.

The FT-IR analysis is pivotal in elucidating the reduction and stabilization mechanism. The spectrum identifies key functional groups from clove phytochemicals, prominently phenolics, flavonoids, and terpenoids, which are known for their redox potential. The intense carbonyl (C=O) stretch at 1636.2 cm^{-1} and the broad hydroxyl (O–H) band at ~3356 cm^{-1} are characteristic of eugenol, the major bioactive component of clove, and other polyphenols. Recent mechanistic studies suggest that the enol/keto tautomerism in molecules like eugenol facilitates the electron transfer required to reduce Ag^+ to Ag^0 (**Singh et al., 2010**). The strong peaks in the 1272–1349 cm^{-1} and 1084 cm^{-1} regions further implicate phenolic acids and polysaccharides. The quantitative peak area data, showing the largest negative areas for the carbonyl (1636 cm^{-1}) and ether/polysaccharide (1084 cm^{-1}) bands, provides compelling evidence that these groups are not merely present but are primary actors in the process. This suggests a dual role: the carbonyl/phenolic groups drive the reduction. Similar results were found by **Khandel et al. (2018)**, who synthesized AgNPs from the lichen *Parmotrema tinctorum*. **Mohammed et al. (2023)** produced AgNPs from an aqueous extract of *Malvaviscus arboreus* leaves. Also, **Abdul-Zahra et al. (2025)** Used the *Triticum aestivum* L . extract as a reducing agent to Ag^+

The EDX analysis showed unequivocal evidence for the formation of elemental silver, with a distinct Ag signal (~3 keV), and the most prominent feature was a broad, intense band centered at 1636.2 cm^{-1}, characteristic of the stretching vibrations of carbonyl groups (C=O) in conjugated ketones, amides, or quinones. This band is frequently associated with flavonoid and terpenoid constituents, which are known to participate in redox reactions. This presence strongly supports the FT-IR findings, confirming that organic phytoconstituents from the clove extract form a capping layer around the AgNPs. This bio-corona is essential for preventing aggregation; therefore, we can deduce that some biological components of the clove extract, such as proteins and metabolites, served a dual purpose of bio-reduction and stabilization because **Abdel-Megeed et al. (2024)** reported that the carbonyl groups in proteins strongly attach to metals, suggesting that proteins may have also formed a layer with bio-organics to protect interactions with phytosynthesized NPs to prevent agglomeration during the synthesis of AgNPs.

The bioassay results clearly demonstrate that enhancing the aqueous clove extract with biosynthesized AgNPs creates a significantly more potent insecticidal formulation. The probit analysis revealed that the AgNP-enhanced extract had a 24-hour LC_{50} of 1.52 ppm, which was 4.34 times more potent than the aqueous extract alone. This enhancement is greater than that achieved by the alcoholic extraction (2.50 ppm, 2.64 times more potent), indicating that the nanoparticle contribution is substantial and distinct from merely improving phytochemical solubility.

The ANCOVA model, explaining 92.5% of the variance, robustly confirms the main effects of formulation, time, and concentration. The lack of a significant extract × time interaction suggests that the relative superiority of the AgNP-enhanced and alcoholic extracts is consistent across the exposure durations tested. The pairwise comparisons confirm that both enhanced formulations are statistically superior to the aqueous extract, with no significant difference between the alcoholic and AgNP-enhanced extracts in this model after adjustment. However, the probit analysis, which models lethal concentration directly, highlights the absolute potency advantage of the AgNP formulation, particularly its significantly lower LC_{50} and LC_{90} values.

The dramatic enhancement of efficacy can be attributed to a synergistic mechanism where the silver nanoparticles (AgNPs) and phytochemicals from the clove extract act in concert. The small size and high surface-area-to-volume ratio of the AgNPs (~28 nm) facilitate superior penetration through the insect cuticle and cellular membranes, simultaneously acting as carriers for the co-localized bioactive compounds like eugenol (**Rai et al., 2018; Ranjani et al., 2020; Deka et al., 2021; Alimi et al., 2023; Rahim et al., 2025**). This enables a multi-target toxicological assault: the clove extract functions as a neuro- and cytotoxin, while the AgNPs contribute additional mechanisms such as reactive oxygen species (ROS) generation and direct binding to critical biomolecules **(Sargsyan et al., 2025; Gwada et al., 2025; Sati et al., 2025**). This combination likely overwhelms the insect's detoxification systems. Furthermore, the phytochemical capping on the AgNPs may allow prolonging the toxic insult compared to the free extract and enhancing the bioavailability and residual activity of biopesticides. Consequently, the observed time-dependent decrease in LC_{50} values aligns with this model, reflecting the cumulative physiological disruption from phytochemicals and the progressive oxidative stress and ion release from the AgNPs.

To the best of our knowledge, this is the first report on the use of silver nanoparticles mediated by clove bud extract to improve the insecticidal efficiency against *Stegobium paniceum*, a drugstore beetle. Nonetheless, the aforementioned findings are consistent with research conducted by other scientists on stored insects and silver nanoparticles derived from different plant species. In a previous work, extracts from *Camelina sativa* were utilized to create silver nanoparticles that, following a 72-hour exposure period, produced death rates of 60.1% against *O. surinamensis* and 46.2% against *S. granarius* at a concentration of 500 ppm (**ur Rehman et al., 2021**). Also, **Al Radadi et al. (2021)** demonstrated that silver nanoparticles made utilizing *Spirogyra hyalina* extract as a capping and reducing agent showed 30% mortality against *T. castaneum* at 500 ppm. In order to demonstrate contact toxicity effects against *T. castaneum* and *S. oryzae* after 24 hours, **Vadlapudi and Amanchy (2017)** used biogenic AgNPs generated from *Myriostachya wightiana* leaf extracts. At a concentration of 50 g, mortality rates of 29% and 20.3% were noted. In contrast, the largest dose (150 g) resulted in a 24-hour death rate of 47.4% for *S. oryzae* insects and 55.2% for *T. castaneum* insects. Furthermore, Using sweet orange extract peels,

Sedighi et al. (2019) generated green-created silver nanoparticles and evaluated their effectiveness. They found that the LC_{50} values of the synthesized AgNPs were 30.62 ppm for the filter paper residue tests against the adult *Tribolium confusum*. **Pushparani et al. (2023)** observed that silver nanoparticles generated from the leaves of *Calotropis gigantea* and *Calotropis procera* had a good insecticidal efficacy against *T. castaneum*. In another study, **Elma et al. (2025)** found that cinnamon-extract-coated silver nanoparticles had the maximum adverse effects at the highest concentration after 72 hours (60.72%), but the aqueous extract of cinnamon had no significant toxic effect on *C. maculatus*. More contact toxicity was shown by biogenic silver nanoparticles (AgNPs) against *T. castaneum* and *S. oryzae* (**Abdel-Megeed et al. 2024**).

CONCLUSION

The structural and bioefficacy results demonstrate the successful phyto-mediated synthesis of spherical, well-dispersed silver nanoparticles (AgNPs) with an average size of 28.34 nm, stabilized by polyphenolic and polysaccharide compounds derived from clove extract. When formulated as an insecticidal agent, this AgNP-enhanced extract exhibited significantly greater potency against *S. paniceum* adults than the standard aqueous extract, as evidenced by a 4.34-fold lower LC_{50} (1.52 ppm at 24 hours) and a statistically significant 20.8% increase in mortality in the ANCOVA model. These findings directly link the defined physicochemical properties of the biogenic nanoparticles to a measurable enhancement in bioactivity, presenting a promising synergistic nano-formulation for pest control. To advance this potential, future research should prioritize field-efficacy trials under field conditions, comprehensive safety and ecotoxicological profiling for non-target organisms and environmental impact, and the exploration of diverse botanical extracts to expand the arsenal of effective nano-biopesticides.

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