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EVALUATION OF INSECTICIDAL POTENTIAL OF SILVER NANOPARTICLES SYNTHESIZED BY USING RIVER RED GUM (EUCALYPTUS CAMALDULENSIS) LEAF EXTRACT

Original Research

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ABSTRACT

Background: Aphids, particularly *Myzus persicae*, are significant agricultural pests, known for their rapid reproduction and ability to cause severe damage to crops, both directly by feeding and indirectly as virus vectors. The extensive use of chemical pesticides to control aphids poses risks to human health and the environment. Nanotechnology, particularly silver nanoparticles, has emerged as a promising eco-friendly alternative due to its potent insecticidal properties.

Objective: This study aimed to synthesize silver nanoparticles using *Eucalyptus camaldulensis* leaf extract and evaluate their insecticidal activity against aphids under controlled conditions.

Methods: Silver nanoparticles were synthesized through the green synthesis approach. Fresh *E. camaldulensis* leaves were boiled in deionized water to prepare the leaf extract, which served as a natural reducing and stabilizing agent. Silver nitrate was added to the extract in a 9:1 ratio, and the formation of silver nanoparticles was confirmed by a color change from pale yellow to dark brown. The nanoparticles were characterized using a BT-90 nano laser particle size analyzer, revealing an average size of 31.5 nm. For the bioassay, the leaf-dipping method was employed, with three concentrations of silver nanoparticles (original solution, 3:1, and 1:1 dilutions) tested on aphids. A control group was treated with deionized water, and mortality was observed over 24 hours. Statistical analyses were conducted using one-way ANOVA and Probit Analysis.

Results: Complete mortality (100%) was observed in all treated groups within 24 hours. The LT50 values for the original solution, 3:1 dilution, and 1:1 dilution were recorded as 11.0 ± 1.67, 12.4 ± 1.85, and 18.6 ± 2.01 hours, respectively. The control group exhibited a minimal mortality rate of 6.7%.

Conclusion: Silver nanoparticles synthesized using *E. camaldulensis* leaf extract demonstrated significant insecticidal activity against *Myzus persicae*, highlighting their potential as an eco-friendly alternative to chemical pesticides.

Keywords: Aphids, biocompatible synthesis, *Eucalyptus camaldulensis*, green nanotechnology, *Myzus persicae*, pest control, silver nanoparticles

INTRODUCTION

Aphids are notorious agricultural pests that feed on plant phloem, transmitting diseases and significantly reducing crop yields. Their destructive impact extends to a wide variety of plants, including vegetables, ornamental species, and commercially significant crops like tobacco. Of the approximately 4,000 aphid species inhabiting temperate regions, only about 100 have become economically significant due to their exploitation of plant hosts. These pests weaken plants by extracting essential nutrients for growth and reproduction while injecting toxic saliva during feeding. They also facilitate the proliferation of sooty mold by excreting honeydew, further impairing photosynthesis. Aphids, such as *Zymus persicae*, threaten nearly 40 plant families, including *Solanaceae*, the family of tobacco (*Nicotiana tabacum*), a crop valued worldwide for its commercial importance(1, 2). The excessive reliance on synthetic chemical pesticides to manage these pests has led to severe environmental and health challenges. These chemicals often harm non-target organisms, including beneficial insects, birds, aquatic life, and soil microbes, while contributing to ecological imbalance. Furthermore, the overuse of pesticides has resulted in the emergence of resistant pest populations, necessitating even higher doses of chemicals, exacerbating toxicity. Pesticide residues on stored grains and crops pose significant risks to human health, increasing mortality rates among agricultural workers exposed to hazardous chemicals. Many pesticides, including organochlorines, carbamates, and organophosphates, have been restricted due to their detrimental effects on both ecosystems and human health, creating an urgent need for alternative, sustainable pest management strategies(2, 3).

Nanotechnology has emerged as a promising frontier in agricultural innovation, addressing the dual challenges of pest management and crop production enhancement. Nanoparticles, with their unique physical and chemical properties, offer an effective solution for pest control due to their large surface area-to-volume ratio, enabling precise interaction with pest surfaces. Among various nanoparticles, silver nanoparticles (AgNPs) have garnered attention for their potent insecticidal properties. By disrupting pest growth and survival, silver nanoparticles demonstrate significant potential for controlling aphid populations. However, conventional synthesis methods for nanoparticles, relying on chemical and physical processes, are often energy-intensive, costly, and environmentally hazardous due to the use of toxic reductants and reactants(4, 5). To address these challenges, researchers are increasingly turning to green synthesis methods as an eco-friendly alternative for nanoparticle production. This approach leverages biological sources such as plants, which are abundant, cost-effective, and environmentally benign. Plant-based synthesis not only eliminates the need for harmful chemicals but also produces stable nanoparticles suitable for diverse applications. In this context, the leaf extract of *Eucalyptus camaldulensis* has been explored as a natural reducing agent for synthesizing silver nanoparticles. This plant-mediated green synthesis method aligns with global efforts to develop sustainable technologies, reducing environmental risks while maintaining efficacy(6, 7). The present study investigates the insecticidal potential of silver nanoparticles synthesized using *Eucalyptus camaldulensis* leaf extract against aphids. This research aims to provide a sustainable, effective, and environmentally friendly solution to mitigate aphid infestations, contributing to improved agricultural productivity and ecological balance(8, 9).

METHODS

The leaves of *Eucalyptus camaldulensis* were freshly collected from Islamia High School, Mohni Road, Lahore. To ensure cleanliness and remove any potential contaminants, the leaves (15 grams) were washed thoroughly with tap water twice, followed by three washes with distilled water. The excess water was removed from the leaf surfaces, and the leaves were cut into small pieces, ranging in size from 0.5 to 1 cm. These pieces were boiled in 100 mL of deionized water, and the extract was allowed to cool to room temperature. The resulting solution was filtered first through a muslin cloth to remove large debris and then through Whatman filter paper No. 01 to achieve a purified extract(10, 11). Silver nanoparticles were synthesized following the reduction of silver nitrate using the method outlined by Bashir et al. (2015). A mixture was prepared by combining 90 mL of silver nitrate solution with 10 mL of the prepared leaf extract in a 9:1 ratio. This solution was stirred using a magnetic stirrer until the color changed from pale yellow to dark brown, indicating the successful reduction of silver nitrate to silver nanoparticles. The presence of phytochemicals in the leaf extract facilitated this process by acting as natural reducing and stabilizing agents for the nanoparticles(12, 13).

The bioassay was conducted using the original silver nanoparticle solution and two dilutions (3:1 and 1:1). Tobacco leaves were treated by dipping them into the respective solutions, ensuring that each leaf absorbed 500 µL of the solution. Leaves treated with deionized water served as the control group. After air-drying, the treated leaves were placed in jars under laboratory conditions, and each jar housed 10 aphids. The aphids were exposed to the treated leaves for 24 hours. Mortality was observed and recorded at regular intervals by

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

gently probing the aphids with a fine brush to detect movement in their legs or antennae, an indicator of survival(14, 15). Statistical analysis was performed using one-way ANOVA with SPSS (Version 16.0) to evaluate the differences between treatment groups. The LT50 (lethal time for 50% mortality) was determined through Probit Analysis using Mini-tab (Version 17.0)(16, 17). The characterization of silver nanoparticles was carried out using a BT-90 nano laser particle size analyzer to record the particle size distribution and confirm the successful synthesis of nanoparticles(18, 19).

RESULTS

The results of the study demonstrated the insecticidal activity of silver nanoparticles synthesized using *Eucalyptus camaldulensis* leaf extract against aphids, with a notable dose-dependent response observed. When exposed to three different concentrations of silver nanoparticles—original solution, 3:1 dilution, and 1:1 dilution—the aphids exhibited complete (100%) mortality within 24 hours. In contrast, the control group treated with deionized water showed a minimal mortality rate of 6.7%. The progressive increase in mortality across time intervals was evident, with the original solution achieving the highest and most rapid impact, followed by the 3:1 and 1:1 dilutions.

Table: *Percent mortality recorded in control and treated groups of aphids after 24 hours treatment at regular time of intervals.*

Groups		**PERCENT MORTALITY RECORDED**					
		After 4 Hours	After 8 Hours	After 12 Hours	After 16 Hours	After 20 hours	After 24 hours
Control Group	(Deionized water)	0	0	0	0	0	6.7
Treated Groups	Original Silver nanoparticles solution	6.7	33.3	50	80	96.7	100
	3:1 dilution	0	23.3	40	56.7	83.3	100
	1:1 dilution	0	10	23.3	36.7	56.7	80

The LT50 values, calculated to measure the time required to achieve 50% mortality, revealed a clear relationship between concentration and efficacy. The LT50 for the original silver nanoparticle solution was 11.01 ± 1.67 hours, while the 3:1 and 1:1 dilutions exhibited LT50 values of 12.46 ± 1.85 hours and 18.57 ± 2.01 hours, respectively. These results indicated that higher concentrations of silver nanoparticles achieved faster insecticidal effects, aligning with the observed mortality trends.

Table: *LT_{50} for aphids against different concentrations of E. camaldulensis Silver nanoparticle solution*

Treatments	**LT_{50}**	**±**	**SE**	**CI**
Original solution of silver nanoparticles	11.0113	±	1.67311	5.43436 to 14.5655
3:1 dilution	12.4600	±	1.85035	6.69045 to 10.4574
1:1 dilution	18.5717	±	2.00564	14.3326 to 24.7761

Note. LT_{50} is lethal time in terms of hours for 50% population's mortality, SE stands for standard error and CI for confidence interval.

Statistical analysis further validated the significant differences in mortality rates between the treated and control groups at different time intervals. After 8 hours of exposure, the original solution showed the highest mean mortality, significantly different from the control and diluted groups ($P = 0.024$). This trend continued after 16 and 24 hours, where the original solution and 3:1 dilution consistently exhibited superior efficacy compared to the 1:1 dilution and the control ($P < 0.001$). The mortality rates for the treated groups increased steadily over time, confirming the time-dependent and concentration-dependent efficacy of the silver nanoparticles. Characterization of the synthesized silver nanoparticles using a BT-90 nano laser particle size analyzer revealed a mean particle size of 31.5 nm, indicative of their nanoscale dimensions and suitability for bioactive applications. The particle size further underscored the efficiency of the green synthesis method employed in this study.

INSIGHTS-JHR
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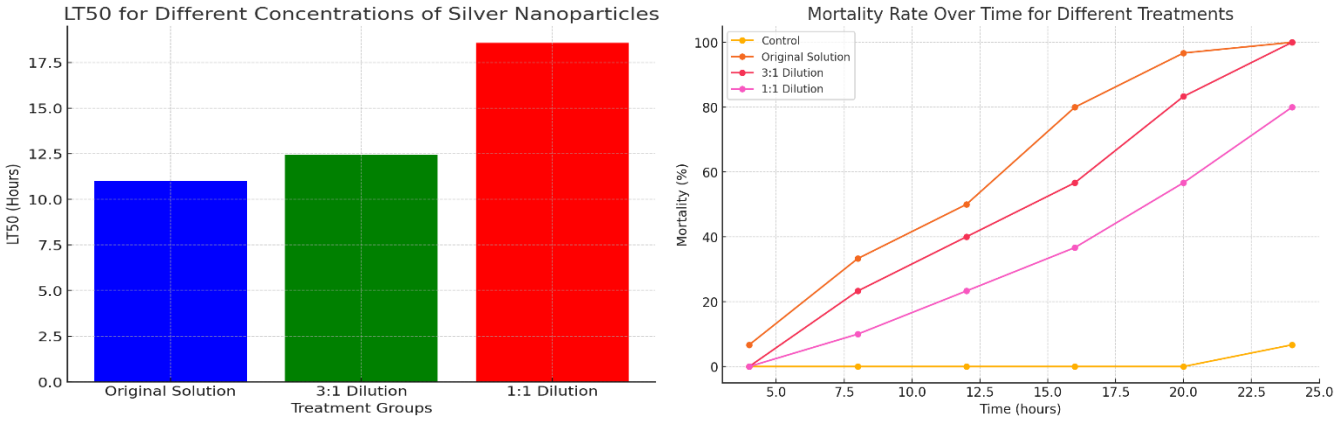
Table: Comparison of Mortality Means Among Control and Treated Groups After 8, 16, and 24 Hours of Treatment

Time (Hours)	Groups	Mean ± SE	df	P-value
8	Control (Deionized water)	$0.000^{b} \pm 0.000$	3,8	0.024
	Original Solution	$3.333^{a} \pm 0.882$		
	3:1 Dilution	$2.333^{ab} \pm 0.667$		
	1:1 Dilution	$1.000^{ab} \pm 0.577$		
16	Control (Deionized water)	$0.000^{a} \pm 0.000$	3,8	0.000
	Original Solution	$8.000^{c} \pm 0.577$		
	3:1 Dilution	$5.667^{ab} \pm 0.882$		
	1:1 Dilution	$3.667^{b} \pm 0.667$		
24	Control (Deionized water)	$0.667^{a} \pm 0.333$	3,8	0.000
	Original Solution	$10.000^{c} \pm 0.000$		
	3:1 Dilution	$10.000^{c} \pm 0.000$		
	1:1 Dilution	$8.000^{b} \pm 0.577$		

Mean ± SE represents the mortality rate of aphids. Superscripts (a, b, c, ab) indicate statistical significance between groups. SE stands for standard error, df for degrees of freedom, and P-value indicates the level of significance.



DISCUSSION

Aphids are among the most detrimental pests in agriculture, causing substantial damage to crops both directly, by feeding on plant tissues, and indirectly, by serving as vectors for plant viruses. Their rapid reproductive potential and ability to infest entire fields pose significant challenges for farmers, affecting both crop yield and quality. Traditional pest control strategies, including the use of chemical pesticides, have proven effective but raise concerns regarding environmental and ecological safety. The development of resistance among pest populations further limits the efficacy of chemical interventions, necessitating the exploration of alternative solutions. Nanotechnology has emerged as a promising innovation in this context, offering environmentally sustainable approaches to pest control while enhancing agricultural productivity (20, 21). Silver nanoparticles, widely recognized for their antimicrobial and insecticidal properties, have gained attention as a potential tool in agriculture. While conventional chemical methods for nanoparticle synthesis are effective, they are often associated with high costs and environmental hazards due to the use of toxic reductants and by-products. Green synthesis, utilizing plant extracts, presents an eco-friendly and cost-effective alternative, leveraging natural phytochemicals as reducing and stabilizing agents. The present study successfully synthesized silver nanoparticles using leaf extract from *Eucalyptus camaldulensis*, resulting in nanoparticles with an average size of 31.5 nm. Variations in nanoparticle size have been observed across studies employing different plant extracts, emphasizing the influence of plant species and phytochemical composition on the synthesis process(21, 22).

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

The insecticidal activity of the synthesized nanoparticles was evaluated against aphids, demonstrating significant efficacy. Complete mortality (100%) was observed within 24 hours for all tested concentrations, with higher concentrations inducing faster mortality. The LT50 values recorded for the original solution and its 3:1 and 1:1 dilutions were 11.0, 12.4, and 18.6 hours, respectively, indicating a concentration-dependent effect. These findings align with previous studies, which have also reported the insecticidal properties of silver nanoparticles against aphids, albeit with variations in efficacy due to differences in synthesis methods and plant extracts used. The use of *Eucalyptus camaldulensis* extract highlights its potential as a viable option for nanoparticle synthesis, contributing to the ongoing exploration of sustainable agricultural practices(23, 24). The present study's strengths lie in its use of green synthesis methods and its successful demonstration of the insecticidal potential of silver nanoparticles. The eco-friendly approach not only reduces environmental risks but also offers a scalable and cost-effective solution for pest management. However, certain limitations warrant consideration. The study was conducted under controlled laboratory conditions, which may not fully replicate the complexities of field environments. External factors such as environmental variability, plant diversity, and aphid population dynamics could influence the efficacy of the nanoparticles. Additionally, the mechanism underlying the insecticidal activity of the nanoparticles, including potential impacts on non-target organisms, remains unexplored and requires further investigation(25).

The findings underscore the importance of green nanotechnology in developing innovative pest control strategies while highlighting the need for comprehensive studies to validate these results under field conditions. Future research should focus on optimizing nanoparticle synthesis methods, understanding the underlying mechanisms of action, and evaluating potential ecological impacts to establish silver nanoparticles as a sustainable and effective tool for integrated pest management.

CONCLUSION

The findings of this study demonstrated that silver nanoparticles synthesized using *Eucalyptus camaldulensis* leaf extract possess significant insecticidal activity against aphids, effectively achieving complete mortality under controlled conditions. This eco-friendly and cost-effective approach highlights the potential of green synthesis as a sustainable alternative to conventional chemical pesticides, offering a promising solution to address pest-related agricultural challenges. The study reinforces the utility of silver nanoparticles as a valuable tool in integrated pest management, paving the way for further research to optimize their application and evaluate their broader impact on agricultural ecosystems.

AUTHOR CONTRIBUTIONS

Author	Contribution
Anwar Khan	Conceptualization, Methodology, Formal Analysis, Writing - Original Draft, Validation, Supervision
Shehzad Sikandar	Methodology, Investigation, Data Curation, Writing - Review & Editing
Samiyah Tasleem	Investigation, Data Curation, Formal Analysis, Software
Shehroz Abdullah	Software, Validation, Writing - Original Draft
Arif Ullah Khan	Formal Analysis, Writing - Review & Editing
Noor Ul Ain Arshad	Writing - Review & Editing, Assistance with Data Curation
Zubeda Bhatti	Writing - Review & Editing, Assistance with Data Curation

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