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INSECTICIDAL ACTIVITY OF GREEN SYNTHESIZED SILVER NANOPARTICLES FROM *PELARGONIUM CITRONELLUM* AGAINST CITRUS MEALYBUG, *PLANOCOCCUS CITRI*

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ABSTRACT

Planococcus citri is a common insect pest that poses a serious threat to citrus crops. Treatment of *Planococcus citri* with chemical pesticides causes harm to humans, animals, and the environment. This study aimed to use silver nanoparticles as potential alternatives to control this agricultural insect pest. Silver nanoparticles (AgNPs) have been identified using X-ray diffraction (XRD), ultraviolet-visible (UV-Vis) spectroscopy, and scanning electron microscopy (SEM). An absorbance peak at 425 nm in the UV-Vis spectrum showed the existence of AgNPs due to plasmon resonance. XRD patterns verified that the AgNPs possess a highly crystalline structure. SEM imaging showed that the AgNPs were spherical in shape with an average size of 12.4 nm. Toxicological tests of the prepared silver nanoparticles showed they are effective in insecticidal activities in different stages of the mealybug. Using the synthesized silver nanoparticles for more than 72 hours of treatment at a concentration of 100 ppm resulted in the highest killing rates of 40.0, 68.0, and 70.0% for the development of three stages of the mealybug, including eggs, the first age of nymphs, and adult females, respectively. The lowest percentage of the insecticidal efficacy of silver nanoparticles was recorded at a concentration of 5 ppm, reaching 62.75 and 4.6 % after 3 and 7 days, respectively. To our knowledge, it is noteworthy to report that nanoparticles were successfully synthesized from the extract of *Pelargonium citronellum* for the first time. The results have demonstrated the potential of natural extracts as a source of nanoparticles to manage mealybugs.

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INTRODUCTION

The order Hemiptera comprises 28 families. One of the most significant agricultural families within this order is Pseudococcidae. This family consists of insect pests characterized by a floury layer and attractive waxy secretions (honeydew secretion) that cover and protect the insects (Avila et al., 2022). One of the most serious agricultural pests related to the family Pseudococcidae is *Planococcus citri* (*P. citri*), called citrus mealybug, which infects various economically important plants worldwide, specifically citrus trees (Irshad et al., 2012; Fateh et al., 2017; Saeed et al., 2019; Bajwa et al., 2020). Their lifestyle has unique features, such as fast reproduction, infecting various ranges of hosts, transmitting plant viruses, and, more importantly, their ability to resist many insecticides (Siddiqui et al., 2023; Ahmed et al., 2023).

For decades, chemicals served as the primary source in insect pest management; however, insects can develop high resistance to synthetic chemicals by coating their body with waxy secretion to protect them from outside harm and prevent it from penetrating their bodies (Ghaffari et al., 2017; Cocco et al., 2020). Furthermore, the extensive use of chemicals as pesticides negatively impacts other beneficial insects and affects the ecosystem balance (Iftikhar et al., 2018). For those reasons, researchers have been focusing on developing eco-friendly management approaches because of the adverse effects of synthetic chemicals on human health and the environment (Shankarganesh et al., 2022; Pathak et al., 2022).

Green synthesis applications occupy a prominent position on the nanotechnology priorities list. The applications of nanotechnology in synthesizing and developing nano-pesticides represent a significant shift in the field of plant protection by providing effective nano-pesticides against most mealybugs and, at the same time, eco-friendless and biodegradable alternatives (Yousef et al., 2023). Management of agricultural pests may soon have a real solution through synthesizing novel nano-pesticides, which can also minimize environmental pollution and reduce application costs, among other issues (Deka et al., 2021; Manna et al., 2023).

The biological synthesis method of silver nanoparticles offers biodegradable and safe substances, reduces environmental pollution, and represents an environmentally sustainable technique (Hassan et al., 2020; Khan et al., 2021). During biological synthesis, the reducing agent plays a dual role in producing and stabilizing the nanoparticles, unlike other synthesis methods requiring specific substances (Dhaka et al., 2023). The production and stabilization of the nanoparticles occurs when biomolecules from the reaction fluid adsorbed on the surface of the nanoparticles, forming the corona. This outer layer improves biological activity and biocompatibility and stabilizes the particles (Hassan et al., 2021; Santos et al., 2021).

Long-term storage conditions of silver nanoparticles (AgNPs) greatly affect their stability. Exposure to daylight and storage of samples at ambient temperature typically induce significant particle alterations. Standard storage conditions may enhance and extend the stability and activity of nanoparticles. Many researchers showed that storage of synthesized silver nanoparticles in the dark at low temperatures (close to 5°C) has long-term stability, with minimal particle agglomeration observed even after more than 100 days of storage (Velgosova et al., 2017).

Silver nanoparticles have been widely applied to address various microbial resistance issues, making them a subject of interest for many researchers (Mohammed et al., 2023; Atiq et al., 2022; Jabbar et al., 2022; Shahbaz et al., 2023). This study aims to biosynthesize silver nanoparticles mediated by *Pelargonium citronellum* leaf extract, for the first time to our knowledge, and emphasize their potential as nano-insecticides for controlling the growth of citrus mealybug.

MATERIALS AND METHODS

Pelargonium citronellum leaf extract

The leaves of *P. citronellum* were collected and cleaned of any dust or particles by washing them three times with distilled water, then they were air-dried at room temperature. Next, they were ground using a small grinder. Ten grams of the powdered *P. citronellum* was weighed and mixed with 100 ml of distilled water, and the mixture was stirred at 40°C for 2 h. The extract was then filtered for purity using Whatman No.1 paper. The resulting extract was stored in an airtight glass vial at 4°C until ready for use.

Silver nanoparticles Fabrication

Silver nanoparticles (AgNPs) were synthesized following the method described in previous studies with some modifications (Salih et al., 2020; Obaid et al., 2023). Briefly, 95 ml of a 1 mM AgNO3 solution was combined with 5 ml of an aqueous solution containing *P. citronellum* extract. Then, the mixture was heated at 60°C on a magnetic stirrer for one hour until the color turned dark brown, indicating the formation of AgNPs.

Silver nanoparticles characterization

The production of AgNPs was confirmed using ultraviolet-visible (UV-Vis) spectroscopy (PG, UK). The size and shape were determined using a field emission scanning electron microscope (FE-SEM, Zeiss, Sigma, Germany). Atomic absorption spectroscopy (Phenox-986AAS, UK) was employed to estimate the concentration of AgNPs. The crystal structure of AgNPs was analyzed with an X-ray diffractometer (Bruker D8 advance, Germany) utilizing Cu Kα1 radiation at 1.5405 A ∘ , 30 kV, and 40 mA in parallel beam geometry. A scan speed of 2 ∘ /min was determined and applied to collect data from 2θ = 10 to 90 ∘ .

Laboratory culture of mealybug *P. citri*

The potato tuber seedling breeding method was utilized to breed the mealybug *P. citri*, as described by Al-Ali (1969). Potato seedlings were artificially infected after the mealybugs were bred on potato tubers. Subsequently, they were cultivated under controlled conditions in a culture room at a temperature of 26 ± 2°C with a relative humidity of 55-60%. The lighting duration (light:dark) was set at 8:16 hours.

Insecticidal activity of AgNPs on *P. citri*

The insecticidal effect of silver nanoparticles was investigated on citrus mealybugs using concentrations of 100, 50, 25, 10, and 5 ppm, prepared through a biological method. Eggs, first-age nymphs, and adult females were separately treated with these concentrations by placing 20 eggs, 10 first-age nymphs, and 10 adults on orange leaves immersed in the respective concentrations. The leaves were then placed in tightly covered plastic Petri dishes with three replicates each. The negative control treatment involved using only deionized water.

The Petri dishes were placed in a culture room maintained at a temperature of 26 ± 2°C, a relative humidity of 55-60%, and a light-dark cycle of 8:16 hours. Killing rates for the mealybug instars were recorded after 24, 48, and 72 h, while eggs that did not hatch after 72 h were considered to have exceeded the typical incubation period and were recorded after 120 h. The killing percentages were calculated using Abbott's equation, and the median lethal concentration (LC50) values were determined using Probit analysis software. Also, a positive control using a silver nitrate solution was included for comparison.

Statistical Analysis

Data were calculated as mean ± standard deviation based on three replicates. A one-way ANOVA test was performed to examine statistical hypotheses, and statistical significance was defined as *p*-values less than 0.05.

RESULTS AND DISCUSSION

Characterization of AgNPs

Pelargonium citronellum extract was utilized as both a reducing and capping agent for the synthesis of silver nanoparticles. This marks the inaugural report on the synthesis of metal nanoparticles utilizing *P. citronellum* extract. Throughout the biosynthesis of AgNPs, the solution transitions in color from a pale yellow to a dark brown, indicative of the presence of AgNPs. The UV-Vis analysis data is depicted in Figure 1, revealing a prominent peak at 425 nm, as illustrated in the spectrum data. This peak corresponds to the surface plasmon resonance peak caused by the oscillations of surface electrons in silver metal within an electromagnetic environment, thus confirming the production of AgNPs from *P. citronellum* extract.

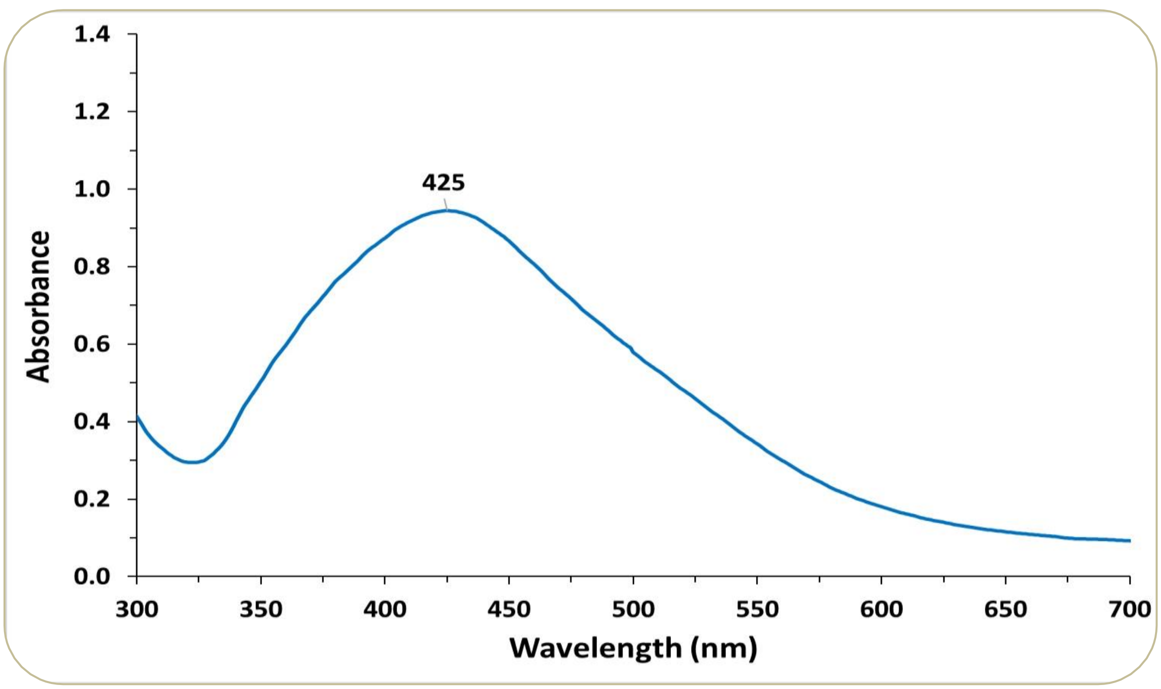

Figure 1. UV-Vis spectrum of silver nanoparticles showing a peak at 425 nm.

The size, morphology, and shape of the synthesized AgNPs were analyzed using FE-SEM. As shown in Figure 2, the results indicate that the AgNPs are evenly scattered and nearly spherical in shape. The average size of biosynthesized AgNPs is between 33 and 50 nanometers. In a prior study, AgNPs of equal size and shape were created using the green synthesis approach with onion peel extract. The investigation indicated the creation of consistent AgNPs that were spherical in shape with an average size of 12.40 nanometers.

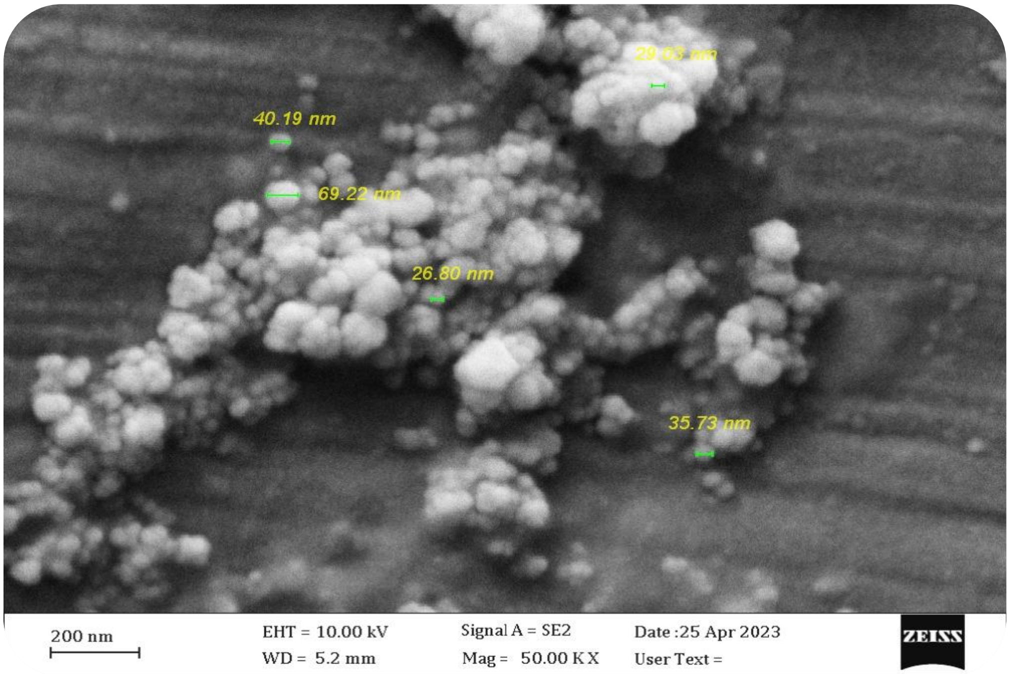

Figure 2. FE-SEM image of AgNPs synthesized from *P. citronellum* extract.

The crystal structure of AgNPs was examined using an X-ray diffractometer. Figure 3 displays the pattern of AgNPs prepared by the biosynthesis method. Two peaks are visible with different intensities. The diffraction angle is noted at (2θ = 38.45), attributed to the Miller index 111, and at (2θ = 68.50), attributed to the Miller index 220. These results indicate that AgNPs were formed, according to JCPDS 04-0783.

Effect of AgNPs on *P. citri* stages

The results depicted in Figures 4 and 5 indicate variations in the insecticidal effectiveness of silver nanoparticles at different stages of mealybugs (adults and nymphs). The highest cumulative killing rates were observed at a concentration of 100 ppm, with rates of 76.7% and 68.0% for adults and nymphs, respectively. Conversely, the lowest cumulative killing rates were recorded at a concentration of 5 ppm. After 72 hours of treatment, the highest rate of killing insect eggs was 40%, as illustrated in Figure 6. Statistical analysis revealed significant differences among the concentrations of silver nanoparticles in terms of lethal rates at a 0.05 level of probability.

Silver nanoparticles have the ability to interfere with several vital activities of insects due to their capacity to penetrate the plasma membrane and degrade numerous essential components, including enzymes. This disruption also affects the coagulation of proteins, impairing the functionality of the plasma membrane and ultimately leading to cell demise (Benelli, 2018; Pathipati and Kanuparthi, 2021). Additionally, exposure of insects to silver nanoparticles reduces larval movement and stiffens the body wall, causing it to bulge, become doughy and brittle, and turn dark brown (Ali et al., 2022). These symptoms resemble those observed in mealybug adults and nymphs treated with various nanoparticles in the current study.

The importance of silver nanoparticles prepared using biological methods in affecting mealybugs could be a significant factor in integrated pest management programs. Metal nanoparticles enhance the attachment of insecticides to the insect's body, leading to cell dehydration and various histological and morphological defects (Nie et al., 2023). In citrus mealybugs, the waxy secretion also aids in adsorbing silver nanoparticles, thereby increasing the insecticide's activity against mealybugs (Zaheer et al., 2022). In another study, it was indicated that silver nanoparticles saturated with carbons damaged the skin and internal cellular tissues (Sultana et al., 2018). Another study demonstrated that the accumulation of ZnO nanoparticles significantly impacted insect organs (Banumathi et al., 2017).

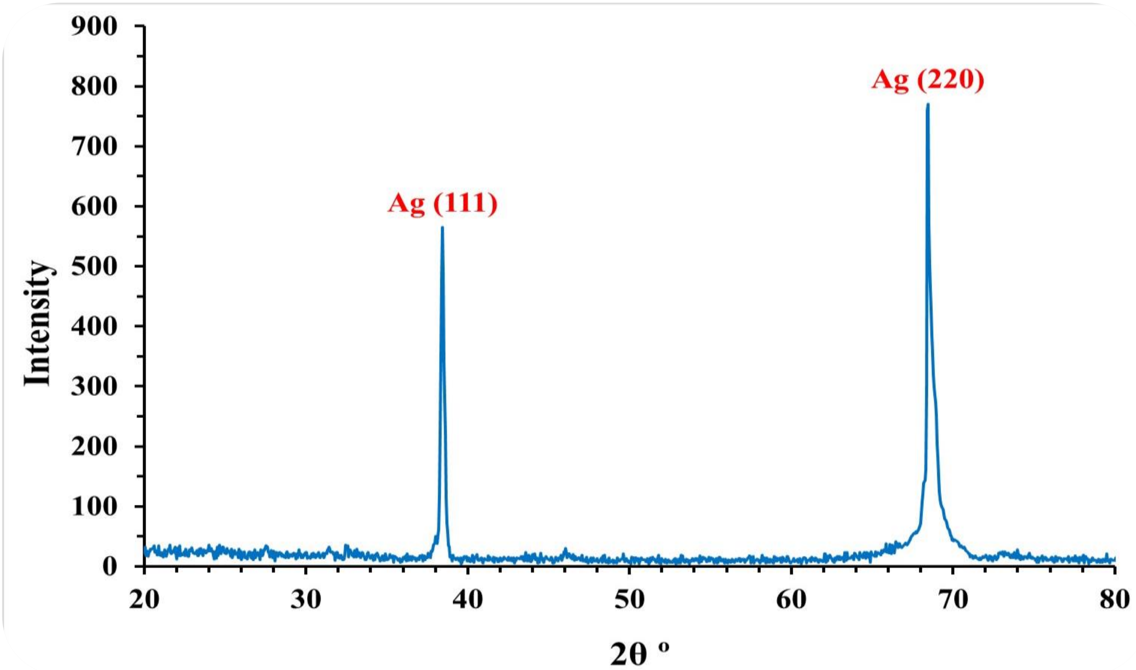

Figure 3. XRD pattern of AgNPs synthesized from *P. citronellum* extract.

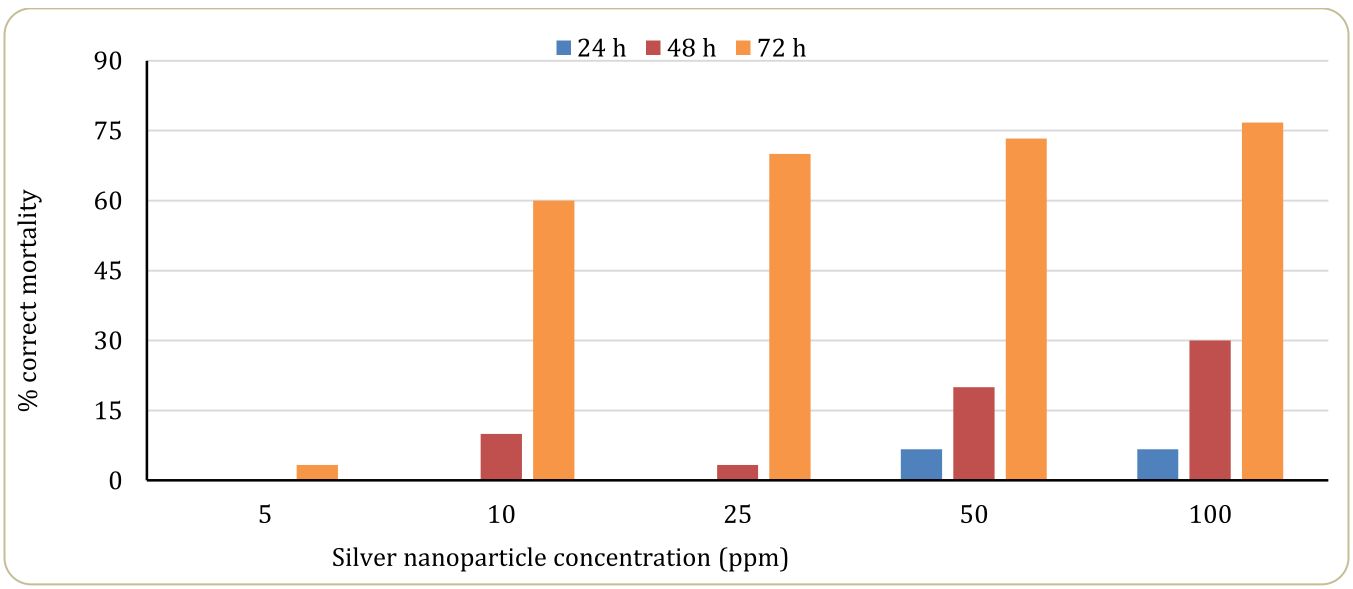

Figure 4. The insecticidal effectiveness of AgNPs in adult female *P. citri.*

The penetration of metal nanoparticles into the insect's body induces oxidative stress, increases DNA damage, and alters physiological processes (Demir, 2020; Chen et al., 2022; Chimkhan et al., 2022). Furthermore, nanoparticles have a significant impact on DNA gene expression when AgNPs and CdNPs are used against the larvae of *Chironomus riparius* (Nair and Choi, 2011).

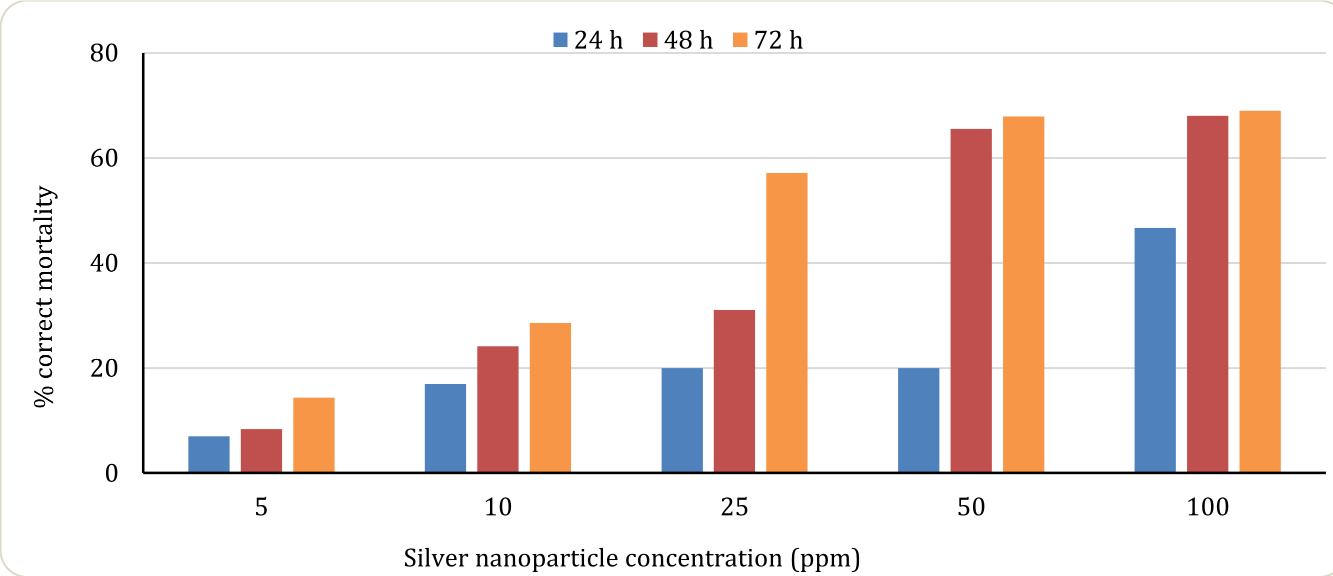

Figure 5. The insecticidal effectiveness of AgNPs in nymphs of *P. citri.*

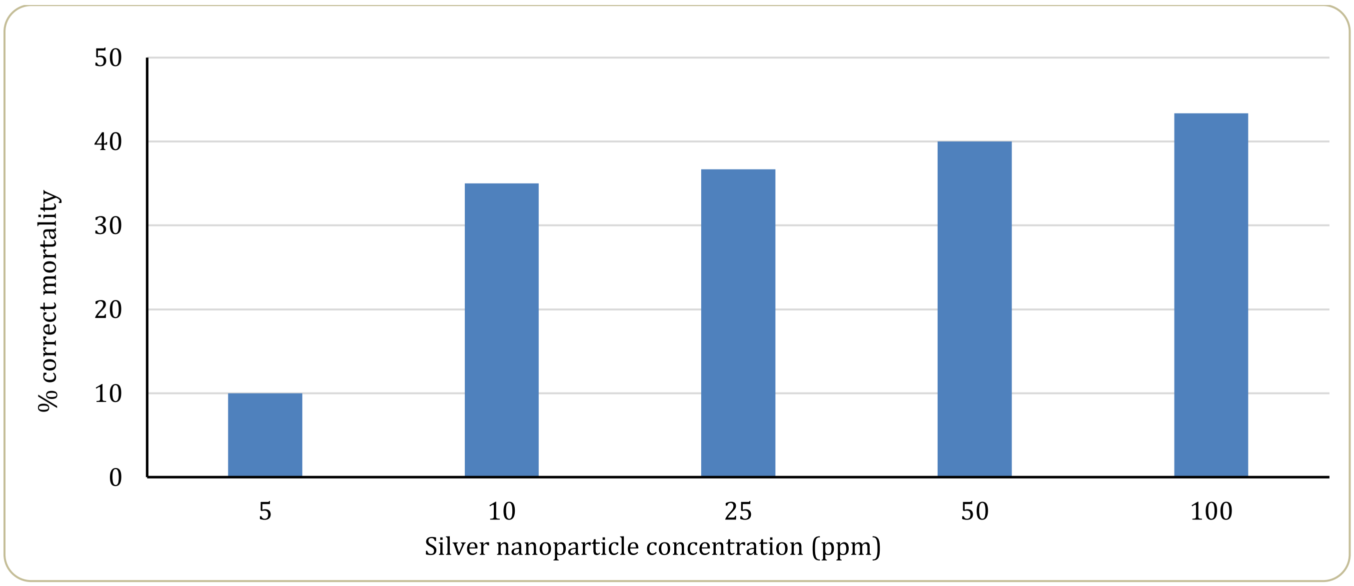

Figure 6. The insecticidal effectiveness of AgNPs in eggs of *P. citri* after 120 h.

Figure 7 depicts a decrease in the insecticidal effectiveness of silver nitrate at various stages of mealybugs (eggs, nymphs, and adult females) compared to silver nanoparticles. The highest killing rate was observed when treated with silver nitrate at a concentration of 100 ppm (13.56%, 21.45%, and 20.0% for eggs, nymphs, and adult females respectively), while the lowest percentage of killing was recorded at a concentration of 5 ppm. The effect of the plant extract of *P. citronellum* was also assessed against different life stages of *P. citri*. It was found that the extract had no effect on mealybug adults, while it exhibited a weak effect on the insect's eggs and nymphs, as detailed in Table 1.

In similar studies, the effect of silver nanoparticles surpassed that of silver nitrate and the aqueous extract of the *Euphorbia prostrata* plant against the rice weevil *S. oryzae*. For instance, a concentration of 250 mg/kg achieved killing rates of 70.0% after 72 h of treatment, whereas the aqueous extract of the seed plant and silver nitrate at a concentration of 1000 mg/L had fatality rates of 40% and 37%, respectively (Zahir et al., 2012). Moreover, a concentration of 10 mg/L of silver nanoparticles resulted in killing rates of 100% for the fourth instar larvae of mosquitoes, whereas it required an increase in the concentration to 50 mg/L of the aqueous extract of *E. prostrata* to achieve killing rates of 83%, indicating the significant superiority of the nanoparticles (Rouhani et al., 2012).

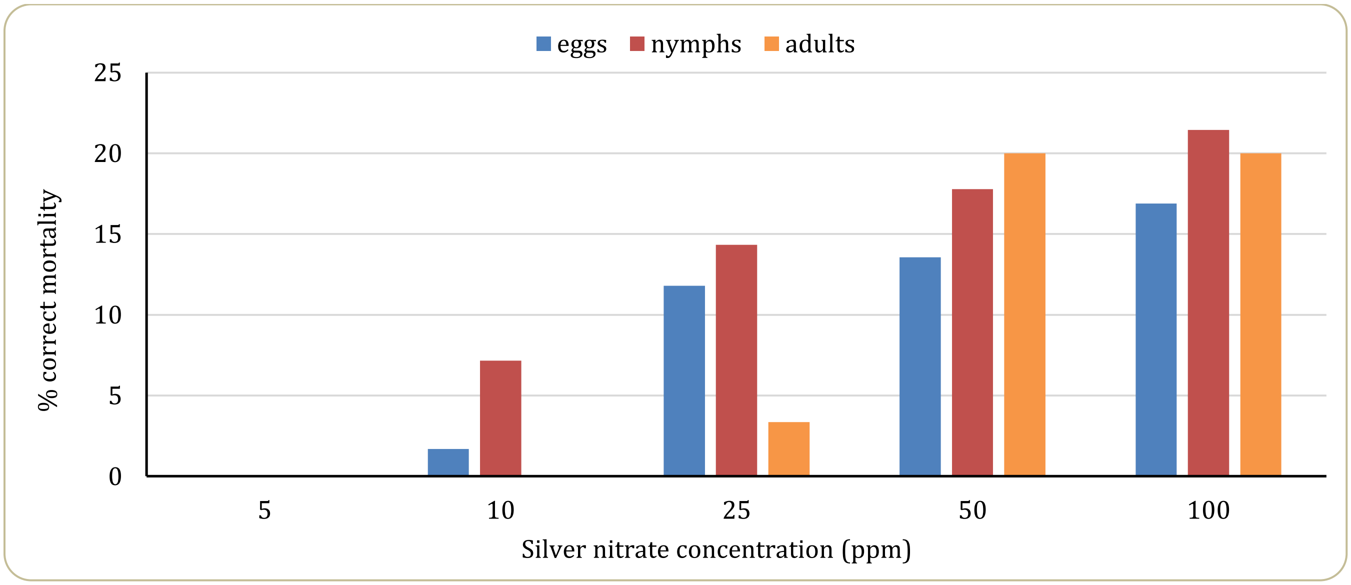

Figure 7. Insecticidal activity of silver nitrate ($AgNO_3$) on life stages of mealybugs *P. citri.*

Table 1. Insecticidal activity of aqueous extract of *P. citronellum* on life stages of *P. citri*

Concentration (mg/ml)	%Corrected mortality		
	eggs	nymphs	adults
5	1.67	0.00	0.00
10	3.33	0.00	0.00
25	6.50	0.00	0.00
50	9.67	3.50	0.00
100	12.33	6.67	0.00

The impact of nanoparticles and their effect on non-targeted organisms, crop plants, and soil has become a major concern in environmental studies, especially when using high concentrations. Many studies have shown that using high concentrations of AgNPs as insecticides has a negative impact on plants across different levels such as morphology, physiology, cellular function, and molecular processes. The varying outcomes suggest the intricate nature of how plants react to AgNPs. These interactions are influenced by characteristic factors of AgNPs (shape, size, chemical form, surface coating, etc.), the specific plant system employed (species, organ, tissue, stage, etc.), and the experimental techniques applied (exposure time, exposure method, medium, etc.) (Yan and Chen, 2019).

However, an analysis of several publications has shown that using appropriate concentrations of AgNPs as insecticides not only manages and controls insects but also enhances the growth of plants. Exposure of the plant to AgNPs enhances the production of reactive oxygen species (ROS) that have various effects on the plant. Increased ROS cause interruptions in physiological processes and damage plant tissue, while low ROS, activated by using low AgNP concentrations, may activate beneficial responses, including defense responses and stimulating plant growth (Chen et al., 2023).

The toxicity level of silver nanoparticles varies according to different factors, including environmental concentration, particulate size, shape, size distribution, and exposure. Therefore, monitoring AgNPs in the environment remains challenging in obtaining accurate and reliable data, even with the progress in nanoparticle synthesis and characterization methods (Yu et al., 2013; Ferdous and Nemmar, 2020; Huang et al., 2022).

CONCLUSION

Mealybugs (*Planococcus citri*) are a serious pest that infects citrus and causes significant economic losses. The current study aimed to evaluate the effectiveness of green-synthesized silver nanoparticles against these insect pests. The study concluded that bio-synthesized silver nanoparticles have insecticidal properties, effectively killing all life stages of mealybugs. Therefore, these nanoparticles are expected to be used as an alternative, environmentally friendly insecticide to protect many other crops against a wide range of agricultural pests.

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AUTHORS' CONTRIBUTION

OMH conceptualized and designed the study; OMH and TYM conducted the experiments, collected and arranged data; OMH analyzed the data; OMH and TYM wrote and proofread the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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