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Research Article

Moringa oleifera based silver nanoparticles: Synthesis and insecticidal toxicity against fall armyworm

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Abstract

The fall armyworm (*Spodoptera frugiperda*) is a major pest of several crops leading to significant yield losses. Developing effective and eco-friendly pest management strategies against fall armyworm (FAW) is crucial for sustainable agriculture. This study investigates the synthesis and evaluation of the insecticidal activity of silver nanoparticles based on an aqueous extract of *Moringa oleifera* leaves (MOL-AgNPs) against FAW. The synthesized MOL-AgNPs were characterized and confirmed using UV-Vis spectroscopy, Energy Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared (FTIR), and Scanning Electron Microscopy (SEM) imaging. Four concentrations (200, 300, 400, and 500 ppm) of MOL-AgNPs and a deionized water as blank control were tested against larval mortality of the last three larval instars of FAW by food dipping method. Results showed different concentrations of MOL-AgNPs exhibited significantly higher larvicidal activity ($p<0.05$). In the 4th instar, 67.5%, 82.5%, and 95% larval mortality were observed in MOL-AgNPs at 500 ppm after 24, 48, and 72 h of the larval exposure, respectively. Similarly in the 5th instar larvae, 72.5% (24 h), 83.75% (48 h), and 90% (72 h) mortality was observed after larval exposures to maximum concentration (500 ppm) of MOL-AgNPs. The 6th instar larvae showed 52.5% (24 h), 57.5% (48 h), and 85% (72 h) percentage mortality at 500 ppm. The MOL-AgNPs demonstrated high larvicidal activity which increased with the increase in concentrations of MOL-AgNPs and duration of exposure. The concentration levels with increased duration of exposure resulted in higher larval mortalities in all three larval instars. However, a more pronounced impact of larvicidal activity was observed on the 4th and 5th instars as compared to the 6th instar. The mortality rates recorded for both the concentration of the nanoparticles and the duration of exposure highlighted the potential of MOL-AgNPs as an eco-friendly and effective pest management strategy.

Keywords: fall armyworm; green synthesis; *Moringa*; pest control

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Introduction

Fall armyworm (FAW) is a serious insect pest of over 350 plant species including several crops of economic importance such as maize, cotton, rice, soybean, sorghum, millet, and sugarcane (Lalramnghaki *et al.*, 2021; Pittarate *et al.*, 2021; Pehlivan and Atakan, 2022; Ali *et al.*, 2023). In maize crop, yield losses of 70% have been reported due to FAW (van den Berg *et al.*, 2021; Abbas *et al.*, 2022; Samanta *et al.*, 2023). Originating from America, FAW expanded its range from Africa to Asia, where it became a major pest of many cultivated crops, particularly affecting maize crop (Montezano *et al.*, 2018; Ramzan *et al.*, 2021; Toepfer *et al.*, 2021; Tahir *et al.*, 2022). The occurrence of FAW in maize crop has been reported in different regions of Pakistan such as Faisalabad (Khan *et al.*, 2015), Multan (Riaz *et al.*, 2024), and Sindh (Gilal *et al.*, 2020; Bhatti *et al.*, 2021; Yousaf *et al.*, 2022). The invasive potential of FAW is intensified due to its high reproduction rate, migration behaviour (Montezano *et al.*, 2018), and adaptability to a wide range of climates and regions (Fonseca-Medrano *et al.*, 2020).

The dependency upon synthetic chemical pesticides for FAW control is effective but has long-term adverse effects (Babendreier *et al.*, 2020). These pesticides disturb the population dynamics of natural enemies and may allow the rapid spread of new pest species expanding host ranges like FAW (Pimentel, 2009; Skendžić *et al.*, 2021; Fareed *et al.*, 2023). Traditional control practices negatively affect non-target organisms, reducing soil biodiversity and pollinators (Kannan *et al.*, 2023). This demands the introduction of novel, environment-friendly, and cost-effective pest management strategies such as using nanoparticles.

Nanomaterials are significant developments in agricultural pest control (Zheng *et al.*, 2020). Plant extracts and nanomaterials offer a favourable source of ingredients capable of producing eco-friendly bio-pesticides as a reliable alternative method for pest control (Khan *et al.*, 2015; Fatima *et al.*, 2016; Qiu *et al.*, 2023). However, challenges exist in tackling their physiochemical characteristics. In this regard, nanotechnology emerged as a crucial tool to tackle this issue effectively (Iqbal *et al.*, 2024). The synthesis of nanomaterials using plant extracts has been widely explored, involving metals such as copper (Lee *et al.*, 2011), zinc (Santhoshkumar *et al.*, 2017), Ti/Ni (Schabes-Retchkiman *et al.*, 2006; Noruzi, 2015), magnesium (Essien *et al.*, 2020), gold and silver (Zuhrotun *et al.*, 2023). Among these, silver nanoparticles (AgNPs) have garnered particular attention due to their low toxicity in humans (Jaswal and Gupta, 2023). Silver nanoparticles (AgNPs) are efficient in drug delivery because of their large surface area, compatibility with the body, easily modifiable, and can make effective entry into the target cells (Xu *et al.*, 2020).

Moringa oleifera (family: Moringaceae) also called as miracle tree has gained recent attention for its versatile applications in agriculture and industry (Anjorin *et al.*, 2010; Shakour *et al.*, 2023). *Moringa oleifera* is rich in secondary metabolites (Pop *et al.*, 2022). Moringa oleifera based silver nanoparticles (MO-AgNPs) have shown exceptional biological and catalytic properties that qualify them for use in medicine as pesticides, antibacterial, anticancer, and catalytic agents (Abdel-Rahman *et al.*, 2022), and antioxidants (Chumark *et al.*, 2008). Studies have suggested that *Moringa oleifera* should have been evaluated to identify and isolate active or synergistic compounds having other than therapeutic potential. Using synthetic insecticides to manage the FAW infestation levels gives local control of the pest but also trade-offs with the issue of insecticide resistance and environmental implications. Thus, the present study assesses the insecticidal potential of MO-AgNPs against fall armyworm larvae.

Materials and Methods

Insect collection and rearing

Three larval instars of fall armyworm (*S. frugiperda*) were used in the bioassay to evaluate the toxicity of *Moringa oleifera* leaves-based silver nanoparticles (MOL-AgNPs). The insects were collected from corn (*Zea mays*) fields adjacent to the Hafiz Hayat Campus, University of Gujrat (2°38′29.55″N, 74°9′55.58″E), Punjab, Pakistan in May, 2024. The identification of FAW was made by using taxonomic keys and published literature (Higo *et al.*, 2022; Widhayasa *et al.*, 2022). Larval rearing was carried out on fresh maize leaves and baby corn. During the bioassay, the temperature (25 ± 1°C), humidity (70 ± 5%) and photoperiod (14h light: 10h dark) were maintained. From the next generation stock, larvae from the 4^{th}, 5^{th}, and 6^{th} instars were randomly selected and larval weight was recorded before the bioassay to ascertain uniformity of treatment variables.

Preparation of leaves aqueous extract

Green and healthy leaves of *M. oleifera* were collected from Kharian (32°49'0"N, 73°51'34"E), thoroughly washed and sun-dried before grinding to make fine powder. The solution was prepared by adding and mixing 100 g of *M. oleifera* leaf powder with 1000 mL of distilled water and kept in the water bath at 50 °C for two hours. Finally, the solution was then centrifuged to collect supernatant (Mohammed and Hawar, 2022).

Biosynthesis AgNPs using M. oleifera

Biosynthesis of AgNPs was prepared by adding and stirring 50 mL of Moringa leaf extract into 50mL of 10mM of $AgNO_3$ (Premasudha *et al.*, 2015). The colour change from pale green to brownish black was observed in the solution and was kept in the dark for 72h at room temperature for colour stabilization (Fareed *et al.*, 2023). The solution was centrifuged to separate solid nanoparticles as a pellet and were left for air-drying to evaporate residual solvent and stored at 4 °C (Mohammed and Hawar, 2022).

Nanoparticles characterization

AgNPs characterization was analyzed by using UV-Vis spectroscopy to assess λmax (UV-1650 PC meter, Japan) from 200 to 800 nm at a resolution of 1 nm at a scan speed of 300 nm/min (Zia *et al.*, 2017). FTIR spectrometer (Nicolet is5 FT-IR, China) was used to perform Fourier transform infrared spectroscopy (FTIR) analysis in the spectral range of 500-4000 cm^{-1} with a resolution of $4cm^{-1}$ (Nayak *et al.*, 2020; Asha *et al.*, 2022). The reflections were observed in the XRD pattern in the wide angle ranging from 20 to 80 (Mehta *et al.*, 2017). The size and shape of synthesized nanoparticles were recorded by Scanning electron microscopy (SEM) using the TESCAN MIRA3 device (Muthukrishnan *et al.*, 2015).

Insecticidal activity bioassay

The insecticidal activities of the MOL-AgNPs nanoparticles were determined by the dipping method under laboratory conditions (El-Gaby *et al.*, 2022). The different concentrations of nanoparticles (200, 300, 400, and 500 ppm) were prepared by diluting with deionized water. These MOL-AgNPs nanoparticles along with a deionizing water as a blank control were used for testing the insecticidal toxicity against FAW larvae. The clean and fresh maize leaves were chopped in equal-sized pieces and dipped in treatment solutions for 20s and air dried.

Then these maize foliage pieces of the same weight were placed into plastic trays (measuring 255 × 160 × 80 mm). Bioassay was conducted on 4^{th}, 5^{th}, and 6^{th} larval instars separately. The experiment was laid out in Completely Randomized Design (CRD) with four replications. Each replicate having 20 larvae of *S. frugiperda.* Insecticidal activity was recorded after 24, 48, and 72h of larval exposure to the treatments.

Larval mortality

Average Mortality was calculated by:

$$\text{Mortality \%} = \frac{\text{No. of dead larvae}}{\text{Total no. of larvae}} \times 100$$

Statistical analysis

The experimental data on larval mortality in different treatment groups of fall armyworm were subjected to statistical analysis by applying analysis of variance ANOVA and Tukey's HSD test for the comparison of means by using Statistix (ver.8.1). The probability value $p < 0.05$ was considered statistically significant with 95% confidence interval. The Lethal Concentration 50 (LC_{50}: the concentration that kills the 50% of test population) and Lethal Concentration 90 (LC_{90}: the concentration that kills the 90% of test population), were used to evaluate the insecticidal activity. The estimation was carried out by probit analysis using the SPSS Statistics 21.

Results

Visual indication of nanoparticles

A noticeable change in the colour of the reaction mixture confirmed the first indication of the synthesis of Moringa leaf-based silver nanoparticles.

UV-Vis spectroscopy

The UV-Vis spectroscopy analysis revealed distinct absorption peaks at the two wavelengths of 200 and 400 nm, representing the poly-dispersity of AgNPs and the surface plasmon resonance (SPR) of silver nanoparticles (Figure 1).

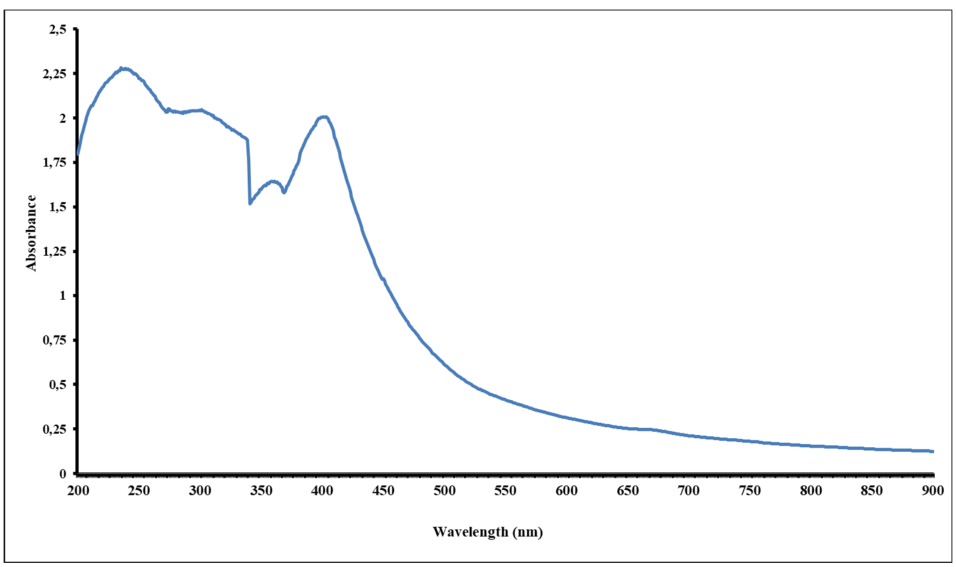

Figure 1. UV-visible spectrum recorded for 10mM $AgNO_3$ with *Moringa oleifera* leaf extract

Scanning Electron Microscopy (SEM)

The SEM analysis confirmed the spherical shape with an average particle size ranging from 30-55 nm confirming the uniformity of the synthesized MOL-AgNPs. The observation of larger nanoparticles could be attributed to the ability of silver nanoparticles to agglomerate (Figure 2).

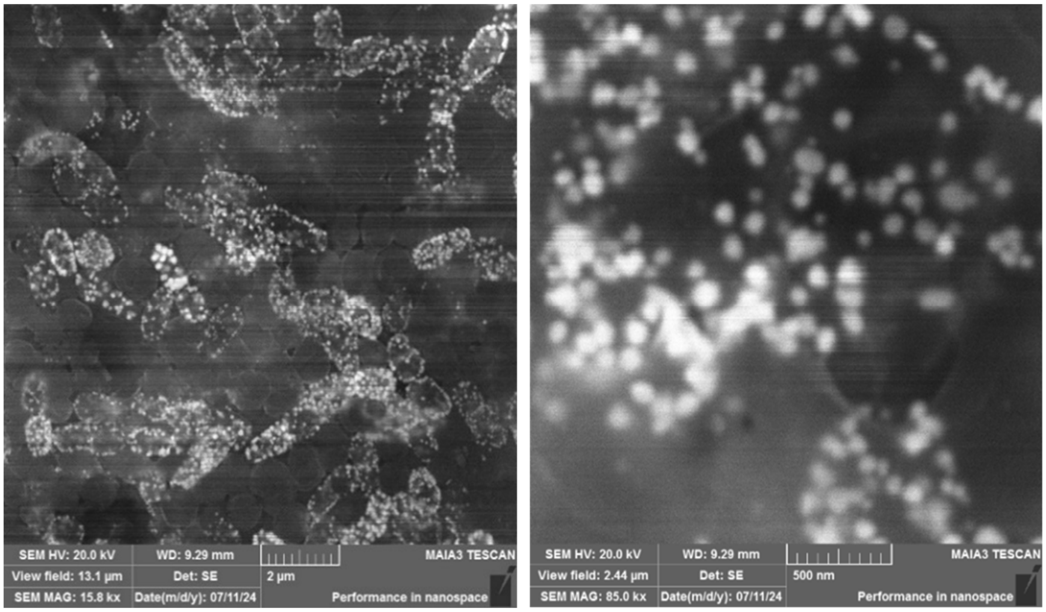

Figure 2. Imaging of MOL-AgNPs through SEM; Left: at 2-micrometer scale, Right: at 500-nanometer scale

Energy Dispersive X-ray spectroscopy (EDX)

The EDX analysis further validated the presence of various elements, with prominent peaks corresponding to the presence of silver ions. This confirmed the successful reduction of silver ions and the synthesis of AgNPs (Figure 3).

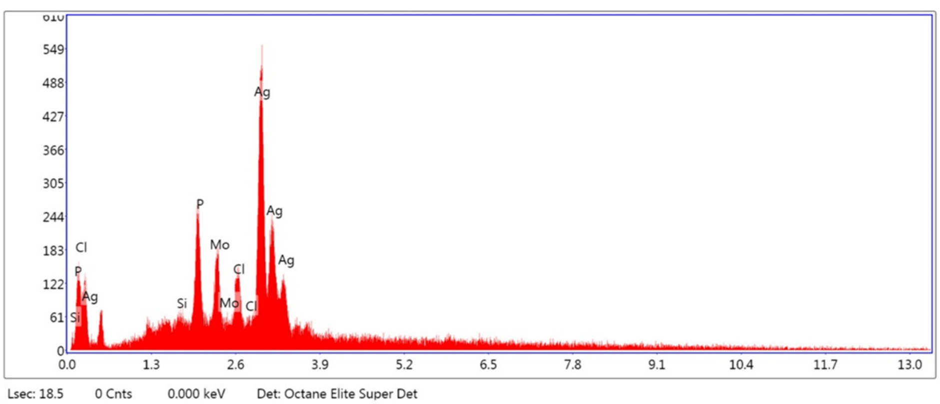

Figure 3. Elemental composition of synthesized MOL-AgNPs through EDX

FTIR results of Moringa leaf extract

The FTIR spectrum of Moringa leaf extract showed distinct peaks at specific wavenumbers. The FTIR spectrum indicated various broad, medium, and strong peaks representing different functional groups in Moringa leaf extract (Figure 4). A broader peek around 3,500-3,200 cm^{-1} shows the presence of hydroxyl groups. The peaks between 3000-1500 cm^{-1} confirmed the presence of alkanes, carbonyl groups, and aromatic compounds.

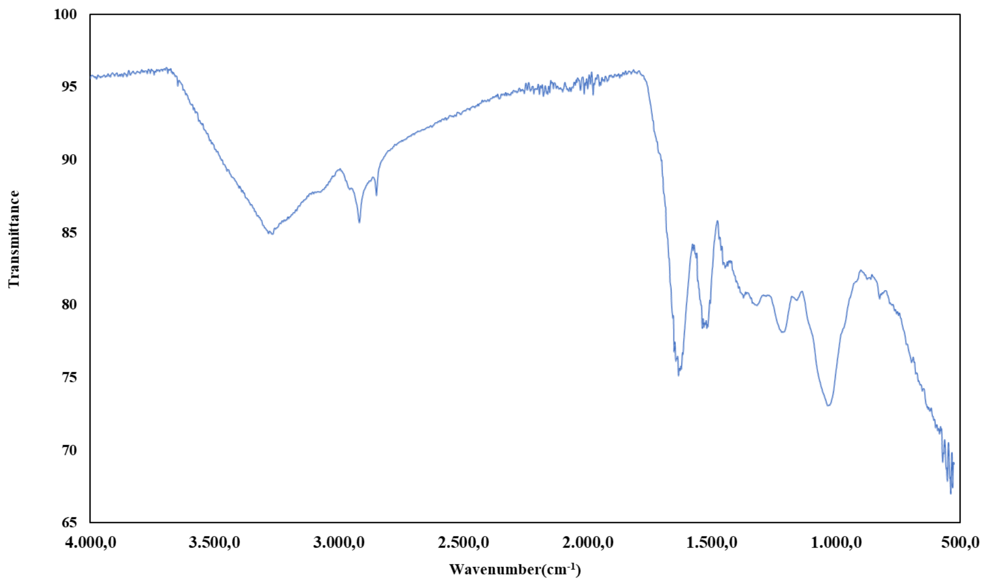

Figure 4. FTIR spectrum of *Moringa oleifera* leaf extract

FTIR results of MOL extract-based AgNPs

The FTIR spectrum of the Moringa leaves extract-based silver nanoparticles revealed distinctive absorption peaks indicative of the organic constituents' present (Figure 5). The peaks between 3,500-1,500 cm^{-1} indicated the presence of O-H, C-H, and C=O stretching which indicated the presence of hydroxyl groups, alkanes, and carbonyl groups. Similar stretching of C=C and C-O around 1,600-1,000 cm^{-1} showed the existence of aromatic compounds and oxygen-containing groups. The comparison of FTIR spectra of both crude Moringa leaf extract and MOL-AgNPs showed that leaf extract provides essential functional groups for the reduction and stabilization of silver nanoparticles. The difference between the spectrums indicates the successful interaction of these functional groups with silver ions, leading to the stable synthesis of AgNPs.

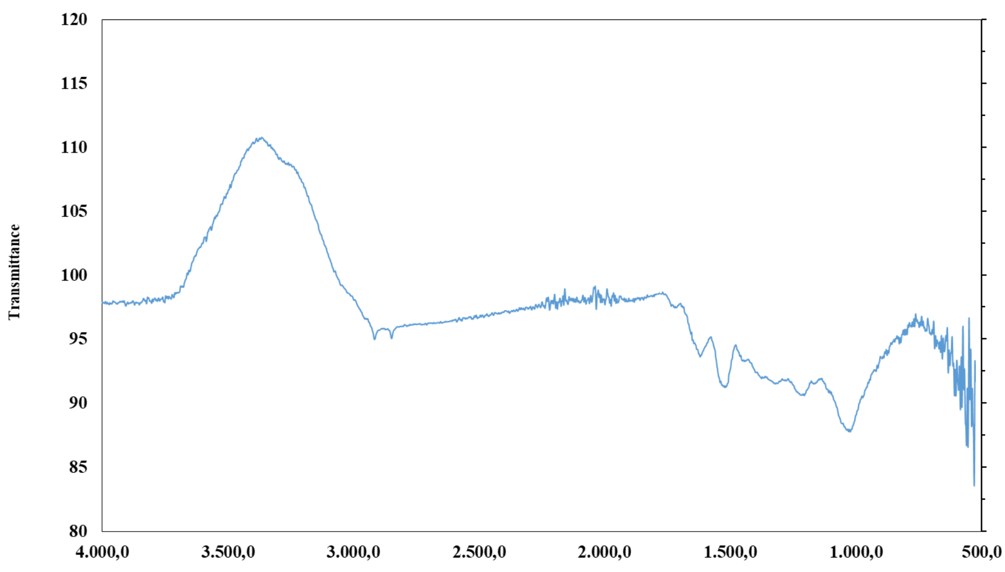

Figure 5. FTIR spectrum of AgNPs synthesized from *Moringa oleifera* leaf extract

Larvicidal activity

The analysis of variance showed significant variations in larval mortality of all instars at ($P<0.05$). The mortality percentage increased with an increase in concentration and exposure time. The Tukey's HSD test results showed significant difference in mean percentage mortality of different treatment groups for all three selected instars (4th, 5th, and 6th instars).

Larval mortality ranged between 1.25-3.75% in the control group after 24h, 48h and 72h, respectively (Table 1). In the 5th instar, larval mortality in the control group also ranged between 1.25-3.75% after 24h, 48h and 72h, respectively (Table 2). In the 6th instar, mortality ranged between 1.25-3.75% in the control group, 6.25-52.5% at 200 ppm, 28.75-68.75% at 300 ppm, 42.5-77.5% at 400 ppm, and 52.5-85% at 500 ppm (Table 3). Data shows a significant increase in the larval mortality which was lowest (3.75%) in control and maximum (95%) at 500 ppm after 72h of exposure (Table 3). Results demonstrated that larval mortality increased with increasing concentration (ppm) of AgNPs and duration of exposure time. The overall highest rate of mortality is 95% at 500 ppm in the 4th instar.

Table 1. Mortality (% ± SE) of 4th instar larvae of fall armyworm

Duration (h)	Concentration (ppm)	Mean ± SE (in %)
24	Control	1.25 ± 1.12 i
	200	17.5 ± 0.79 h
	300	43.75 ± 0.91 fg
	400	57.5 ± 0.65 de
	500	67.5 ± 0.75 cd
48	Control	2.5 ± 0.96 i
	200	35 ± 1.35 g
	300	52.5 ± 0.91 ef
	400	68.75 ± 0.5 cd
	500	82.5 ± 1.15 ab
72	Control	3.75 ± 0.59 i
	200	52.5 ± 0.74 ef
	300	65 ± 0.83 cde
	400	77.5 ± 0.74 bc
	500	95 ± 0.41 a

Table 2. Mortality (% ± SE) of 5th instar larvae of fall armyworm

Duration (h)	Concentration (ppm)	Mean ± SE (in %)
24	Control	1.25 ± 1.82 h
	200	28.75 ± 0.79 g
	300	45 ± 0.91 ef
	400	63.75 ± 1.58 cd
	500	72.5 ± 1.02 bc
48	Control	2.50 ± 0.64 h
	200	36.25 ± 0.87 fg
	300	57.5 ± 1.22 de
	400	67.5 ± 0.39 cd
	500	83.75 ± 0.77 ab
72	Control	3.75 ± 4.74 h
	200	47.5 ± 1.26 ef
	300	66.25 ± 0.52 cd
	400	71.25 ± 0.75 bc
	500	90 ± 0.43 a

Table 3. Mortality (% ± SE) of 6th instar larvae of fall armyworm

Duration (h)	Concentration (ppm)	Mean ± SE (in %)
24	Control	1.25 ± 2.04 g
	200	6.25 ± 3.23 g
	300	28.75 ± 3.23 f
	400	42.5 ± 3.54 e
	500	52.5 ± 2.39 de
48	Control	3.75 ± 2.39 g
	200	13.75 ± 1.25 g
	300	43.75 ± 2.39 e
	400	55 ± 3.23d e
	500	57.5 ± 3.23 cd
72	Control	3.75 ± 3.23 g
	200	52.5 ± 1.25 de
	300	68.75 ± 2.39 bc
	400	77.5 ± 1.25 ab
	500	85 ± 1.25 a

The overall mean percentage mortality of FAW larvae when exposed to different concentrations (200, 300, 400, 500 ppm, and Control) of MOL-AgNPs showed maximum mortality at 500 ppm in all three larval instars i.e., 4th, 5th and 6th instars (Figure 6 a-c).

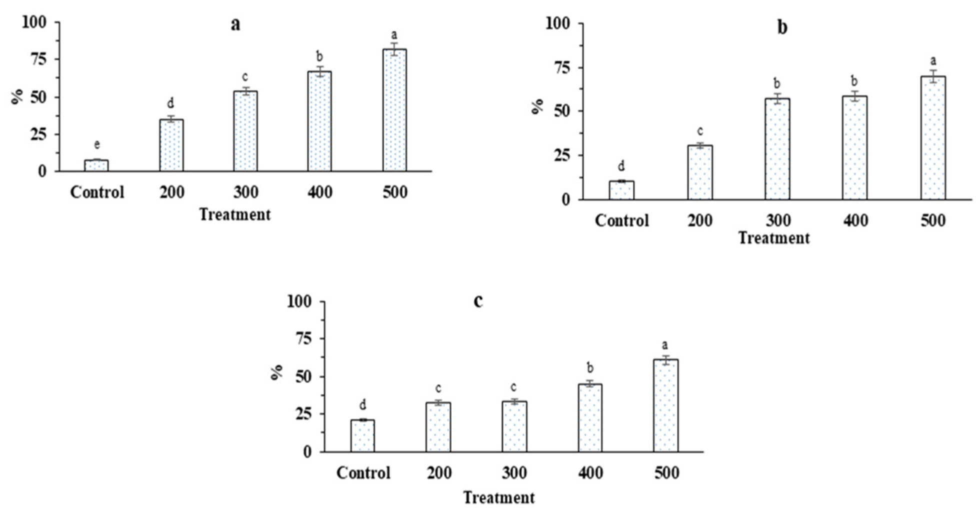

Figure 6. Overall mortality (%) of (a) 4th, (b) 5th and (c) 6th instar larvae of FAW in treatments of MOL-AgNPs solutions

Probit analysis

Probit analysis was performed to calculate the lethal concentrations (LC_{50} and LC_{90}) of synthesized MOL-AgNPs against FAW. The LC_{50} and LC_{90} values indicate the concentrations required to kill 50% and 90% of the population, respectively. LC_{50} and LC_{90} values were determined using probit analysis via SPSS ver.21. The lethal concentration (LC_{50} and LC_{90}) values and chi-squared (χ^2) values for the 4th, 5th, and 6th instars of FAW exposed to MOL-AgNPs for 24, 48, and 72h were calculated (Table 4).

Table 4. Lethal concentration values (LC_{50} and LC_{90}) calculated in probit analysis for different instars at different time intervals

Instar	Duration (h)	LC_{50} (ppm)	LC_{90} (ppm)	χ^2
4th	24	2.56	2.94	6.09
	48	2.44	2.83	4.44
	72	2.31	2.71	9.90
5th	24	2.6	3.18	7.70
	48	2.49	3.09	10.2
	72	2.37	2.75	7.06
6th	24	2.65	3.06	6.28
	48	2.55	2.9	4.19
	72	2.44	2.77	6.84

In the 4th instar, the LC_{50} values decreased from 2.56 ppm to 2.31 ppm (362 to 206.7 ppm) as the duration of larval exposure increased from 24 to 72h. The 5th instar larval mortality showed a similar trend, with LC_{50} values decreased from 2.6 ppm to 2.37 ppm (236.4 to 401.8 ppm) after the larval duration increased from 24 to 72h, and LC_{90} values decreased from 3.18 to 2.75 ppm (1527 to 573.2 ppm). The response of 6th instar larvae to the concentration of MOL-AgNPs follows the same pattern i.e., LC_{50} values decreasing from 2.71 to 2.44 ppm (522.3 to 279.3 ppm) after duration of exposure from 24 to 72h, and LC_{90} values decreasing from 3.06 to 2.77 ppm (1120.4 to 597.5 ppm). This trend indicates that longer exposure times result in lower concentrations needed to achieve lethal effects, demonstrating the time-dependent efficacy of MOL-AgNPs.

The chi-squared (χ^2) values ranged between 4.19 to 10.2 across the all three instars and duration of exposure. Compared to the critical value for 14 degrees of freedom, which is approximately 23.68 at a significance level of 0.05, all χ^2 values are within the threshold (Table 4). The line is relatively steep in all the probit graphs at three-time intervals, indicating a strong relationship between the log of concentration and the probit response of the 4th (Figure 7), 5th (Figure 8), and 6th larval instars of FAW (Figure 9). A steep slope indicated high potency, meaning lower concentrations are effective. This suggests that increase in the concentration of MOL-AgNPs leads to a significant increase in the probit value.

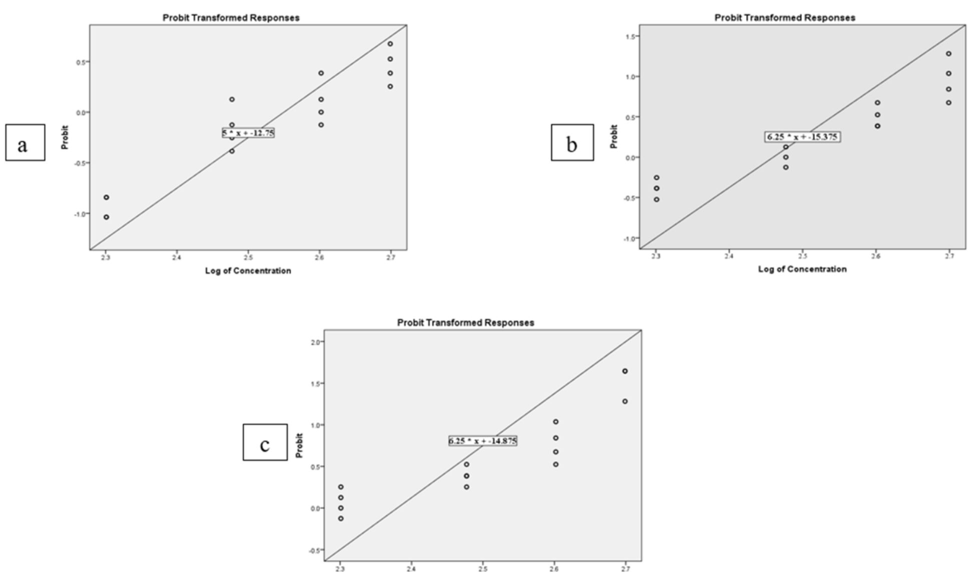

Figure 7. The probit graph illustrating the relationship between MOL-AgNPs concentration on lethal effect on the 4th instar of *S. frugiperda* after (a) 24 h, (b) 48 h & (c) 72 h

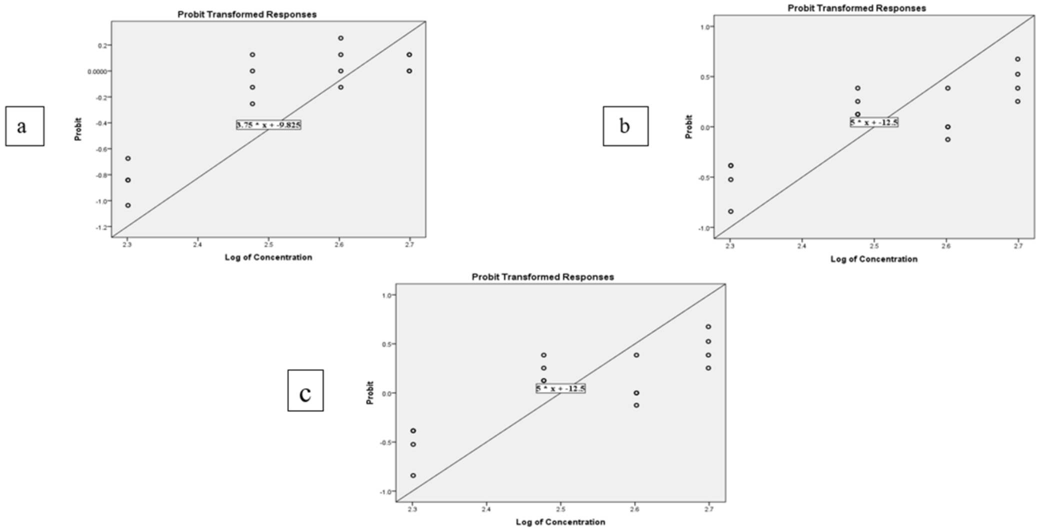

Figure 8. The probit graph illustrating the relationship between MOL-AgNPs concentration on lethal effect on the 5th instar of *S. frugiperda* after (a) 24 h, (b) 48 h & (c) 72 h

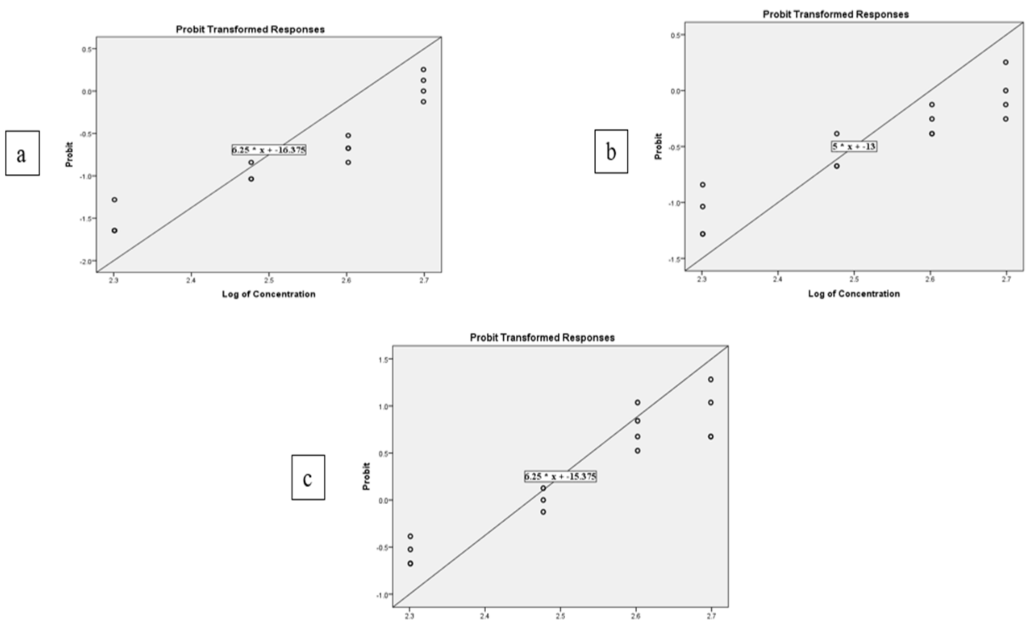

Figure 9. The probit graph illustrating the relationship between MOL-AgNPs concentration on lethal effect on the 6th instar of *S. frugiperda* after (a) 24 h, (b) 48 h & (c) 72 h

Discussion

Green synthesis of MOL-AgNPs exhibited significant insecticidal activity FAW larvae under laboratory conditions. The reaction mixture initially displayed a pale greenish colour, which gradually turned dark brown after 72h, indicating the formation of silver nanoparticles (Neethu *et al.*, 2018). The synthesized MOL-AgNPs were characterized by Fourier Transform infrared (FTIR), UV-visible Spectroscopy, Scanning Electron Microscope (SEM), and Energy Dispersive X-ray (EDX). The FTIR spectrum of prepared nanoparticles was similar to the results reported earlier wherein observed absorption peaks were at 3,328 cm^{-1}, 2,919 cm^{-1}, 2,951 cm^{-1}, 1,643 cm^{-1}, and 1,010 cm^{-1}. The physical characteristics of MOL-AgNPs were also following the studies conducted earlier reporting the particle size of MOL-AgNPs (ranged between 5 nm to 80 nm). Whereas the average size of nanoparticles was 46 nm, and strong surface plasmon resonance was also recorded at 360 and 460nm (Das *et al.*, 2013). The results of UV-Vis spectroscopy analysis indicated variations which are due to their high surface energy and surface tension. Therefore, minor agglomeration in the micrograph could be attributed to these properties (Aouf *et al.*, 2024). The SEM analysis demonstrated spherical shape with an average particle size. This showed the stability of synthesized silver nanoparticles, facilitated by the active components present in Moringa leaves (Sathyavathi *et al.*, 2011; Abdel-Rahman *et al.*, 2022). Moreover, the minor peaks in the EDX analysis of phosphorous, chlorine, etc. are associated with potential residuals from the Moringa leaf extract (Aouf *et al.*, 2024).

The spherical shape and size range (30-55 nm) of the biosynthesized MOL-AgNPs facilitate the penetration activity. The insecticidal or larvicidal characteristics of nanoparticles could be ascribed to their structure, size, and high covering capacity, which helps their penetration into the insect's body (Sankar and Abideen, 2015).

The higher concentrations and longer exposure times of AgNPs have shown higher insecticidal activity against fall armyworm larvae. Our results follow the pattern earlier reported by studying the impact of Green Chiretta (*Andrographis paniculata*) based nanoparticles used against FAW. The findings demonstrated that the aqueous leaf extract and silver nanoparticles synthesized from *A. paniculata* showed increased mortality rates at different concentrations of both the aqueous leaf extract and the synthesized AgNPs (Rajkumar and Alaguchamy, 2022).

The larval mortality observed in the 4^{th} exhibited variable responses to the concentrations and duration of exposures. For example, maximum mortality was observed in the 4^{th} instar after 72h of exposure at 500 ppm of MOL-AgNPs treatment, which was higher as compared with the 5^{th} and 6^{th} larval instars. Similarly, in the 5^{th} instar larvae, larval mortality increased with an increase both in concentration and duration of exposure. However, the mortality response decreased compared to 4^{th} instar larval mortality. In the 6^{th} larval instar, FAW indicated a lower response of larval mortality as compared to younger larval instars.

The larval mortality percentage may be attributed to several factors such as uptake and interaction, mode of action, and cumulative toxicity of nanoparticles (Tuncsoy and Tuncsoy, 2023). MOL-AgNPs may be efficiently taken up by the larvae through surface contact; leading to internal accumulation and disruption of physiological processes crucial for larval development and survival (Qiu *et al.*, 2023). Silver nanoparticles have shown antimicrobial properties which could disrupt cellular integrity, and may cause cellular toxicity ultimately leading to larval mortality (El-Samad *et al.*, 2022). AgNPs can reduce acetylcholinesterase activity, impairing nerve function (Benelli, 2018). These actions collectively impair the development, reproduction, and larval survival (Shahzad and Manzoor, 2021).

The decreasing LC_{50} and LC_{90} values over time indicated a time-dependent enhancement in the toxicity of MOL-AgNPs. The probable reasons for this trend could be bioaccumulation, sensitivity of the developmental stage, and exposure duration to AgNPs (Wāng *et al.*, 2024). Over time, larvae may accumulate higher concentrations of MOL-AgNPs within their bodies. This accumulation surpasses lethal thresholds more quickly (Benelli, 2018; El-Samad *et al.*, 2024). Different larval instars (4^{th}, 5^{th}, and 6^{th}) exhibit different metabolic rates that depends on age, and surface-to-volume ratios (Chown *et al.*, 2007; Pathipati and Kanuparthi, 2021). This made their vulnerability different towards the applied concentrations of MOL-AgNPs. Younger instars succumb to lower concentrations of nanoparticles more readily (Shahen *et al.*, 2020). These findings also underscore that younger larvae (4^{th} instar) are more susceptible to MOL-AgNPs, with decreasing effectiveness as larvae mature (Murray and Fogarty, 2019). This is clear if we compare the mortality rates of the 4^{th} and 5^{th} instar stages with the 6^{th} larval stage of fall armyworm. The cuticular proteins that are stage-dependent play an important role in providing insects with insecticidal resistance (Fatima *et al.*, 2016).

The presence of a thick protein layer provided extra protection to the 6^{th} larval instar as compared to the immature stages of FAW (Zhu *et al.*, 2021). The resistance in FAW is also due to detoxification enzymes, which break down the insecticides, allowing the FAW to survive despite the pesticides. The main families of these detoxification enzymes are P450s, Carboxylesterases, and Glutathione-S-Transferases (Carvalho *et al.*, 2018). The minor resistance shown by the 6^{th} instar larvae could be due to multiple mechanisms; increased activity of enzymes that break down insecticides (detoxifying enzymes) and less sensitivity of important enzymes like acetylcholinesterase towards insecticides (Hafeez *et al.*, 2021; Ranganathan *et al.*, 2022). The LC_{50} and LC_{90} (Lethal concentration for 50% and 90% of the population) for synthesized bio-pesticide increased with larval age. This means older larvae (6^{th} instar) need higher doses of insecticides to be killed compared to younger larvae. Their increased body weight and age increased their tolerance (Haq *et al.*, 2022; Ranganathan *et al.*, 2022; Fareed *et al.*, 2023).

The chi-squared (χ^2) values further validate the results of bioassay. All χ^2 values fall below the critical value of 23.69 (significance level of 0.05) which suggests that the observed data fit well with the expected model. The consistency among results confirmed the MOL-AgNPs efficacy against FAW. The regression analysis showed that the mortality of FAW larvae across all three instars revealed varying efficacy of MOL-AgNPs. Overall, MOL-AgNPs consistently influenced mortality across instars; their efficacy varies, highlighting the importance of developmental stages in pest management strategies.

Conclusions

The synthesis and characterization of *Moringa oleifera* leaf extract-based silver nanoparticles showed insecticidal activity against the 4^{th}, 5^{th}, and 6^{th} instar larvae of FAW. Larvicidal activity of MOL-AgNPs varied significantly between different concentrations and durations. The lower LC_{50} and LC_{90} values of MOL-AgNPs against 4^{th}, 5^{th}, and 6^{th} larval instars indicated their acute toxicity against FAW. The LC_{50} and LC_{90} values decreased with the increased exposure duration of larvae to different concentrations of MOL-AgNPs across all three instars. The results of bioassay suggest MOL-AgNPs have great insecticidal potential against FAW.

Authors' Contributions

AN, MFM and MH conceived idea and designed the research work. AN, UZ and AI conducted the research. MFM and MH supervised the experimental work, performed statistical analysis, and critically reviewed the manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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