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Green Synthesis, Characterization and Larvicidal Activity of Molybdenum Nanoparticles Synthesized Using Acalypha Wilkesiana Against Aedes Aegypti Larvae: A Potential Integrated Mosquito Control Strategy

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ABSTRACT

In tropical countries, where Aedes aegypti is a major vector of arboviral infections including dengue, Zika and yellow fever, mosquito-borne diseases continue to present significant public health challenges. The growing resistance of mosquitoes to conventional chemical insecticides necessitates the development of environmentally sustainable alternatives. The green synthesis, physicochemical characterization and larvicidal assessment of molybdenum nanoparticles (MoNPs) made with Acalypha wilkesiana leaf extract as a natural reducing and stabilizing agent are reported in this work. Fourier Transform Infrared Spectroscopy (FTIR) and UV-visible (UV–Vis) spectrophotometry were used to characterize the biosynthesized MoNPs. UV-Vis analysis revealed a characteristic surface plasmon resonance band at 211 nm, confirming nanoparticle formation, while FTIR spectra identified Mo=O and Mo–O–Mo vibrational modes indicative of molybdenum oxide structures. Aedes aegypti eggs were hatched to larvae which were reared and exposed from their 2nd instar larval stage to MoNPs at varying concentrations in accordance with the World Health Organization (WHO) susceptibility protocol. Larvicidal bioassays against Aedes aegypti larvae demonstrated dose- and time-dependent mortality, with a maximum mortality of 48% recorded at an ultra-low concentration of 0.00125 ppm after 72 hours of exposure. Significant differences ($P < 0.05$) were found between concentrations and times of exposure according to statistical analysis. Although MoNPs did not reach the World Health Organization threshold for full susceptibility, their measurable larvicidal activity highlights their potential as eco-friendly components of integrated mosquito control strategies.

Keywords: Green synthesis, Molybdenum nanoparticles, Acalypha wilkesiana, Larvicidal activity, Aedes aegypti, UV-Vis spectroscopy, FTIR, Vector control, Susceptibility test, World Health Organization

1. Introduction

Worldwide, mosquito-borne illnesses continue to be a leading source of morbidity and mortality, especially in tropical and subtropical areas. Aedes aegypti is known to be the primary vector of dengue fever, yellow fever, chikungunya and Zika virus and mosquito-transmitted diseases account for a significant percentage of hospital admissions in Nigeria[1-3]. The increased use of chemical pesticides has led to environmental contamination, insecticide resistance and negative impacts on creatures that are not the intended target[4]. These difficulties highlight the pressing need for sustainable, environmentally friendly and alternative methods of controlling mosquitoes[4].

Due to the special physicochemical characteristics of nanoparticles, such as high surface-area-to-volume ratios and increased biological reactivity, nanotechnology has become a viable vector control method[5,6]. Among several synthesis techniques, green synthesis, which uses plant extracts, provides an economical, environmentally friendly and biocompatible substitute for traditional chemical pathways. Phytochemicals that serve as stabilizing and reducing agents, such as flavonoids, tannins and phenolics, are utilized in plant-mediated synthesis[5].

Acalypha wilkesiana, a medicinal plant rich in bioactive compounds, has been successfully employed in the biosynthesis of metal nanoparticles[7,8]. However, studies investigating the larvicidal potential of green-synthesized molybdenum nanoparticles remain limited. Molybdenum oxide nanoparticles exhibit redox activity and catalytic properties that may disrupt larval physiological processes[9]. Thus, the purpose of this study was to create molybdenum nanoparticles using leaf extract from Acalypha wilkesiana, describe their physicochemical characteristics and assess how effective they were at killing Aedes aegypti larvae.

2. Materials and Methods

2.1. Study area

The study was carried out in the Nigerian state of Nasarawa, in the city of Lafia **(Figures 1 and 2)**. The population of Lafia, which is located between latitudes 829′N and longitudes 830′E, is primarily agrarian. The danger of diseases spread by mosquitoes is increased by agricultural practices and areas that retain water, which provide ideal circumstances for mosquito reproduction[10].

2.2. Preparation of plant material

Acalypha wilkesiana fresh leaves were gathered, properly cleaned with double-distilled water and allowed to air dry. After being cleansed, five grams of the leaves were chopped into tiny pieces and cooked for two hours in 100 milliliters of double-distilled water. To provide a clear stock solution for the synthesis of nanoparticles, the extract was chilled and filtered through Whatman filter paper (185 µm)[11,12].

2.3. Green synthesis and characterization of molybdenum nanoparticles

Wu, et al.[13] used proven green synthesis techniques to create molybdenum nanoparticles utilizing Acalypha wilkesiana leaf extract as a reducing agent. The creation of nanoparticles was seen by looking for a discernible color shift in the reaction mixture[14-16]. Using UV-visible spectrophotometry, which scans absorbance across the ultraviolet spectrum, nanoparticle formation was verified. Functional groups involved in stabilizing nanoparticles and verifying the formation of molybdenum oxide were identified using FTIR spectroscopy[17].

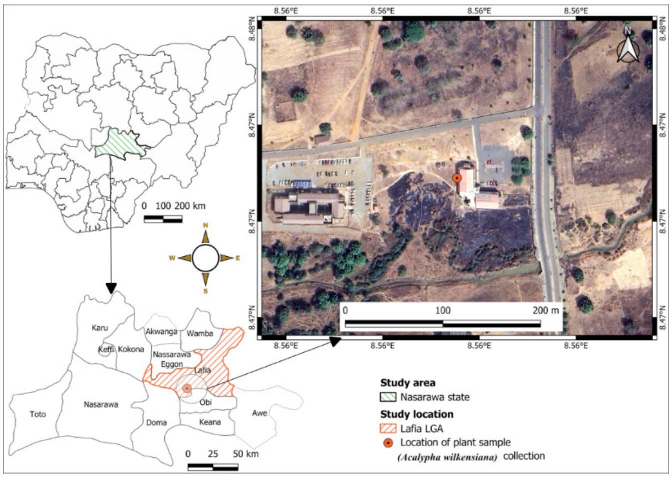

Figure 1: Plant Sample Collection Site in Lafia LGA of Nasarawa State in Central Nigeria (Generated using QGIS version 3.40.1-Bratislava).

Figure 2: Acalypha wilkesiana Plant (Field Photo).

2.4. Collection and hatching of aedes aegypty eggs

Eggs of Aedes aegypti were ordered from the National Arbovirus and Vectors Research Centre (NAVRC), Enugu State, Nigeria. The egg processing protocol as described by Joshua, et al.[18], was adopted. The egg stripes were soaked to hatch within 12-24 hours into a nutrient broth, which was prepared by extracting nutrient broth from agar powder, by adding, mixing and completely dissolving 13g of nutrient agar powder (CM0001B) in 1L of distilled water and allowing the agar residue to settle to get our pure nutrient broth; poured into a conical flask and sterilized by autoclaving at 121°C for 15 minutes. The larvae hatched were fed by adding a pinch of finely powdered yeast and biscuit on the surface of the water.

2.5. Larvicidal bioassay

Larvicidal activity of the synthesized MoNPs was evaluated against *Aedes aegypti* larvae at concentrations of 0.00125, 0.0125, 0.125, 1.25, 12.5, 25, 37.5, 50, 62.5 and 87.5 ppm. A total of 25 mosquitoes (*Aedes aegypti)* larvae were introduced to each of the concentrations and each concentration had four (4) replicates. Also, there were two bowls containing 100 % distilled water and 25 *Aedes aegypti* larvae each, which served as a control. The larvae were fed with compounded yeast and cabin biscuit twice daily. Larval mortality was assessed during exposure periods of 24, 48 and 72 hours and the rate at which larvae were knocked down was recorded at 10, 15, 20, 30, 40, 50 and 60 minutes. The larvae's mortality was confirmed by lightly touching their abdomen with a small needle[19].

2.6. Test inference

The interpretation of the mortality rate of mosquito larvae was based on the guidelines by World Health Organization[20,21] as follows:

- WHO guidelines classify mosquito populations as susceptible when mortality is ≥98% at the diagnostic time and concentrations.
- Possibly resistant when mortality is between 90-97%.
- While considered resistant when mortality is <90%.

2.7. Determination of percentage mortality

Mortality was calculated using Abbott's formula. Non-mobile and moribund larvae were recorded as dead.

$$\% \text{ Mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae exposed}} \times \frac{100}{1}$$

2.8. Statistical analysis

Data obtained from the larvicidal bioassays were statistically analysed using SPSS version 27. The Chi-square (χ^2) test was employed to compare mortality rates across varying concentrations and exposure durations. The level of significance was set at $P < 0.05$ to determine statistically meaningful differences between treatments.

3. Results

3.1. UV-visible spectroscopic and FTIR analysis

UV-Visible spectroscopy of the biosynthesized MoNPs revealed a strong surface plasmon resonance band at 211 nm, indicating that molybdenum oxide nanoparticles have formed **(Figure 3)**. FTIR spectra of the synthesized MoNPs displayed characteristic absorption peaks at 1620.78 cm^{-1} corresponding to O–H bending vibrations, 1121.90 cm^{-1} associated with Mo–O–Mo stretching, 939.36 cm^{-1} attributed to Mo=O stretching and approximately 466.77 cm^{-1} corresponding to Mo–O–Mo bending vibrations. These peaks confirmed the formation of crystalline molybdenum oxide nanoparticles stabilized by phytochemicals from *Acalypha wilkesiana* **(Figure 4)**.

3.2. Larvicidal activity of molybdenum nanoparticles

Molybdenum nanoparticles exhibited moderate larvicidal activity against *Aedes aegypti* larvae in a dose- and time-dependent manner. After 24 hours, mortality ranged from 0–10% across concentrations. At 48 hours, mortality increased, reaching up to 43% at 0.00125 ppm. The highest larvicidal activity was observed at 72 hours, with a maximum mortality of 48% recorded at 0.00125 ppm. Statistical analysis revealed significant differences ($P < 0.05$) across concentrations and exposure periods, particularly at 0.00125 ppm, 0.0125 ppm, 1.25 ppm, 12.5 ppm and 62.5 ppm after 72 hours **(Table 1)**. Despite this activity, mortality levels did not reach the World Health Organization susceptibility threshold.

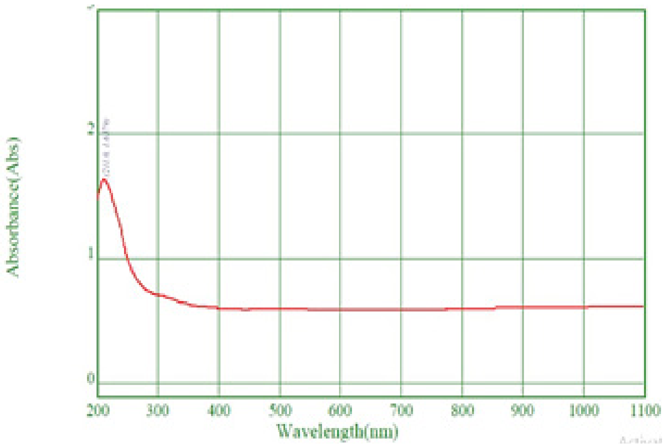

Figure 3: UV-Vis Spectra of the Synthesized AW-MoNPs.

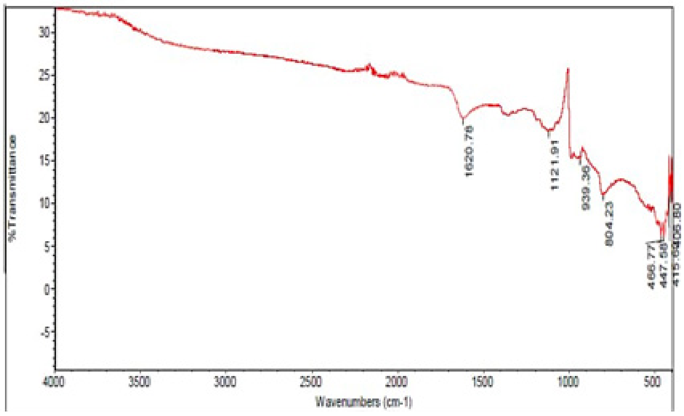

Figure 4: FTIR Spectra of the Synthesized AW-MoNPs.

Table 1: Mortality rate of Aedes aegypti Exposed to Molybdenum NP.

Exposure Period (hrs)	% Mortality Across Concentrations (ppm)													
	0	0.00125	0.0125	0.125	1.25	12.5	25	37.5	50	62.5	87.5	χ^2	df	P-value
24	0	6	4	2	3	0	0	4	9	10	3	25.098	9	< 0.05*
48	0	43	40	11	27	12	28	26	19	17	20	43.132	9	< 0.05*
72	0	48	42	12	28	13	30	26	19	17	20	50.529	9	< 0.05*

*: Significant

4. Discussion

This study successfully recorded green synthesis of molybdenum nanoparticles using *Acalypha wilkesiana* leaf extract as confirmed through UV-Visible and FTIR spectroscopic analyses. The UV-Visible band at 211 nm in this research reveals that the molybdenum oxide nanoparticles (MoO_x NPs) are ultrasmall and well-formed at favourable nano-form highly dispersible across wide surface area and reactive yielding enhanced contact with the larvae via better adhesion to cuticle, penetration into gut tissues and disruption of cellular ion balance. This is in agreement with previous literatures who stated that a strong, sharp UV band indicates small, highly dispersible NPs with large surface area, which generally enhances contact with larvae and release of ions or reactive species[22-24]. Green-synthesized metal/metal oxide NPs with well-defined UV–Vis peaks (for instance, 250–350 nm for MgO, ZnO, Ag) consistently show better larvicidal performance than the corresponding plant extracts alone, due to nano-size and surface reactivity[25-28]. The surface plasmon resonance (SPR) band at 211 nm is consistent with reported molybdenum oxide nanoparticle formations, while the presence of Mo=O and Mo–O–Mo vibrational modes confirm crystalline molybdenum oxide structures[29-31].

Our research highlighted that mortality was minimal at 24 hours but increased significantly by 48–72 hours, reflecting threshold-dependent and prolonged exposure effects, although mortality levels did not reach the World Health Organization susceptibility threshold of ≥98%[21]. These findings align with earlier reports on silver and zinc oxide nanoparticles, which similarly demonstrated strong larvicidal activity through enzyme inhibition, oxidative stress and tissue damage[32]. The observed larvicidal activity of MoNPs against *Aedes aegypti* larvae demonstrates their biological reactivity and toxicological potential. However, the higher mortality observed at lower concentrations, particularly 0.00125 ppm after prolonged exposure, suggests nanoparticle reactivity rather than concentration alone influencing toxicity[33,34]. Furthermore, the larvicidal assay revealed a non-monotonic, inverse dose–response relationship, with peak mortality (48%) at the lowest tested concentration (0.00125 ppm) and declining efficacy at higher doses. This pattern deviates from the conventional monotonic increase in toxicity with dose but aligns with documented non-linear responses in nanoparticle toxicology and aquatic entomology. Key mechanisms likely include NP aggregation at higher concentrations, hormetic/biphasic biological responses and physicochemical stability factors[35-41].

Aggregation is a primary driver of reduced toxicity at elevated NP concentrations in aqueous media as also reported by Narayanan, et al.[36], Rad & Cheng[37] and Römer, et al.[42]. Increased particle density promotes homoaggregation via van der Waals forces and reduces colloidal stability, forming larger agglomerates that sediment rapidly, decrease the surface area-to-volume ratio and limit bioavailability to target organisms, such as mosquito larvae[43-45]. This phenomenon has been extensively reported for metal and metal oxide NPs in aquatic systems. For instance, metal oxide NPs aggregate more readily at higher concentrations in natural aqueous matrices, reducing effective surface reactivity and toxicity[46]. Similarly, gold NPs exhibited smaller aggregate sizes and greater biological interactions at low concentrations (1-20 µg/L), whereas higher doses led to larger aggregates and diminished reactivity[47]. Silver NPs showed attenuated cytotoxicity and reduced cellular uptake in aggregated states, as clumped particles interact less efficiently with biological membranes[48-51]. Metal oxide NPs, including TiO_2 and Al_2O_3, displayed reduced toxicity at higher exposures due to increased hydrodynamic diameters from aggregation, which restricted cellular uptake and surface-mediated effects[52]. In this present study, ultra-low MoNP concentrations likely maintained optimal dispersion (facilitated by plant-derived capping agents from Acalypha wilkesiana), maximizing larval exposure and mortality. Higher concentrations may have induced aggregation, sedimentation and reduced bioavailability, resulting in the observed inverse trend. Future characterization via dynamic light scattering (DLS) across concentrations would confirm this mechanism.

Again, hormesis characterized by low-dose stimulation (or enhanced toxicity) and high-dose inhibition may offer another plausible explanation. In NP systems, low concentrations may induce targeted cellular disruption (e.g., oxidative stress or membrane damage), while higher doses trigger adaptive defences such as upregulated antioxidant enzymes or detoxification pathways, mitigating overall toxicity[39]. Meanwhile, hormetic responses are well-documented in NP-exposed aquatic invertebrates and insects. For example, non-monotonic dose–responses, including increased effects at low doses, have been observed in aquatic organisms exposed to nanoparticle-based toxicants or biopesticides[35,40,41]. In Daphnia magna (a model for aquatic invertebrate toxicity relevant to mosquito larvae), low-dose exposures to certain compounds elicited stronger adverse effects than intermediate or high doses, potentially via adaptive physiological mechanisms[53,54]. Similar biphasic patterns occur in NP toxicology, where low doses promote oxidative stress without overwhelming repair systems, whereas higher doses activate protective responses[55,56]. Therefore, mosquito larvae may exhibit analogous adaptations at elevated MoNP exposures, including enhanced detoxification metabolism, which reduces mortality relative to ultra-low doses that exploit vulnerabilities without triggering defences.

Again, additional factors include suspension stability and experimental artifacts, as also reported in a recent study by Kirubakaran, et al.[57]. Green-synthesized NPs rely on phytochemical capping for dispersion; at low concentrations, these agents effectively prevent aggregation, whereas saturation at higher doses promotes instability, sedimentation or altered surface chemistry, diminishing toxicity[38]. Supporting evidence includes aged or diluted NP suspensions showing enhanced larvicidal activity due to improved dispersion[58,59]. In mosquito control contexts, NP efficacy varies with water chemistry, pH, ionic strength and capping stability, often favouring low-dose performance[60].

The enhanced bioactivity of MoNPs may be attributed to their high surface-area-to-volume ratio and redox-active molybdenum oxide phases, which can induce oxidative stress and disrupt larval physiological processes[61,62]. However, the inability of MoNPs to achieve complete larval mortality indicates that, when used alone, they may not be sufficient as standalone larvicides. Nonetheless, its moderate efficacy highlights the potential role it has in integrated vector management strategies, even though may show more potential particularly when combined with other environmentally friendly control measures.

5. Conclusion

This work establishes the successful green synthesis of molybdenum nanoparticles (MoNPs) using Acalypha wilkesiana leaf extract, confirmed through UV–Visible and FTIR spectroscopic analyses. The detection of a surface plasmon resonance band at 211 nm and the presence of Mo=O and Mo-O-Mo vibrational modes validate the formation of crystalline molybdenum oxide structures. Biologically, MoNPs demonstrated larvicidal activity against Aedes aegypti larvae, but with a distinctive non-monotonic, inverse dose–response relationship. Mortality was highest at ultra-low concentrations (0.00125 ppm) and declined at higher doses, indicating that nanoparticle reactivity, dispersion stability and biological adaptation mechanisms are more critical determinants of toxicity than concentration alone. This unusual pattern can be explained by aggregation at elevated concentrations, which reduces bioavailability and surface reactivity, as well as hormetic responses where low doses induce oxidative stress and membrane disruption while higher doses trigger adaptive detoxification pathways. Suspension stability factors also play a role, as phytochemical capping agents maintain dispersion at low concentrations but become less effective at higher doses, promoting sedimentation and diminished activity. These findings align with broader nanoparticle toxicology literature, where inverse dose–responses and hormesis are increasingly recognized in aquatic entomology and environmental nanoscience.

Overall, the moderate larvicidal efficacy of MoNPs highlights their potential as eco-friendly agents in integrated vector management strategies, though they may not be sufficient as standalone interventions. Their enhanced bioactivity is attributed to high surface-area-to-volume ratios and redox-active molybdenum oxide phases, which can induce oxidative stress and disrupt larval physiology. However, the inability to achieve complete mortality underscores the need for optimization of nanoparticle concentration, formulation and stability to maximize efficacy while minimizing unintended ecological effects. Future studies should employ dynamic light scattering (DLS) to confirm aggregation behavior across concentrations and investigate larval detoxification pathways to better understand adaptive responses. In practical terms, MoNPs could be strategically deployed in combination with other environmentally sustainable mosquito control measures, offering a novel, plant-mediated nanotechnology approach to combat mosquito-borne diseases. This research contributes to the growing evidence that green-synthesized nanoparticles can serve as innovative biocontrol agents, provided their unique dose–response behaviors are carefully considered in real-world applications.

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