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Larvicidal and antioxidant activity of green silver nanoparticles synthesized using *Musa acuminate* peel extract against *Aedes aegypti*

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Abstract
The present study pertains to the exploitation of biowaste generated through Banana (*Musa acuminate* L.) peels for the synthesis of green silver nanoparticles (AgNPs) and their bioactivity was evaluated for managing the dengue vector, *Aedes aegypti*. UV-VIS specrtophotometery was used for confirming AgNPs synthesis and a peak was observed at 426 nm. Characterization of AgNPs was done using Scanning electron microscopy which depicted spherical shape of AgNPs while Transmission electron micrographs revealed their size to be ranging from 20 to 50 nm. AgNPs bioactivity was studied against III instar larvae following 24, 48 and 72 h exposure and values of LC_{50} and LC_{90} were analyzed using probit analysis. LC_{50} values ranged from 10.69 to 25.15 ppm with AgNPs at various time exposures. Antioxidant potential of these AgNPs was also evaluated to supplement these findings. This study highlights the potential of biowastes as a potent larvicide against *Aedes aegypti*.

Keywords: Silver nanoparticles, *Aedes aegypti*, *Musa acuminate*, larvicidal activity

1. Introduction

Mosquitoes are responsible for the spread of many diseases like dengue fever, yellow fever, chikungunya, and Zika virus and all these diseases are caused due to the bite of female *Aedes aegypti*. In the last two decades, extensive research focus is paid for managing dengue at global level as this arboviral disease is proving deadly. Initially, dengue was a native disease of Africa but later expanded to the tropical and subtropical regions of the globe. The most common reason being that dengue occurs in low-income countries because there is no sizeable or predictable market available. Since the last decades, climate change, population growth, deforestation, habitat invasion, and insecticide resistance have contributed to the emergence, re-emergence, and dispersion of several diseases. A major challenge of public health which is affecting hundreds of millions of lives every year on our planet [1]. Currently, no common commercial vaccine or drug is available against dengue viral infection, therefore, the alternative prevention method is mainly achieved through mosquito population control. The most commonly used tool is the application of chemical insecticides and despite their effectiveness, indiscriminate use of these chemicals has led to high operational costs, population resistance and adverse effect on the non-target organisms. Therefore, we need to develop new biodegradable insecticides of paramount importance for future mosquito control strategies.

According to the present scenario, the relevance of nanotechnology emerged as an escalating field of research which involves synthesis design, fabrication, characterization, device formulation and several medical applications such as diagnosis, treatment, drug delivery, medical device coating etc. due to their size and shape. Recent advancements in nanotechnology led to the development of drugs and medicines towards the treatment and prevention therapies of life- threatening diseases. Presently, researchers are interested in formulating large scale production method of solid waste through bio-synthesized metal nanoparticles like silver, gold, and platinum. In recent years, plant-derived larvicides are being reported by many researchers [2,3,4,5] that usually contain a combination of several phytopotentials that work synergistically, targeting different biological processes and therefore, reducing the likelihood of resistance development in their targets.

Few studies were conducted using bilberry and red currant wastes as a source of reducing and stabilizing agents for producing Ag-NPs as they were rich in phenolic compounds and other substances [6, 7, 8]. Recently Soto *et al.* [9] synthesized silver nanoparticles from fruit peels waste against pathogens. These studies were the motivation for the present study, where green silver nanoparticles were synthesized using biowaste from *Musa acuminate* (Banana) peels rich in phenolic compounds and tested for their bioactivity in controlling mosquito vector of dengue.

2. Materials and methods

2.1 *Aedes aegypti*

Aedes is a genus of mosquitoes originally found in tropical and subtropical zones. The *Aedes aegypti* can transmit the viruses that cause dengue fever. *Aedes aegypti* is a dark mosquito with white lyre shaped markings and banded legs. It is considered to be a primary vector of viral diseases such as chikungunya, dengue fever and yellow fever. III instar larvae were selected for evaluating the larvicidal potential of silver nanoparticles.

2.2 *Musa acuminate* L.

Musa acuminate L. is commonly known as Banana, belong to the genus *Musa*. They are mostly found in the tropical regions of Southeast Asia. Banana peel has now been realized as a resource for a number of purposes including organic fertilizer, Banana charcoal, in the treatment of pimples, warts and skin related products, repellent properties, etc. In the present study, it was used as a bioreducing agent for nanoparticle synthesis.

2.3 Preparation of aqueous peel extract

25% aqueous extract was prepared from Banana peels after being washed with purified water. Finely chopped peels were mixed with MilliQ water (100 ml) and gently heated. The extract was filtered through Whatman filter No. 1 and stored in refrigerator for further study.

2.4 Green synthesis of silver nanoparticles

For green synthesis, a microwave assisted protocol was used [10] where 1 mM aqueous silver nitrate ($AgNO_3$) was prepared in a 250ml of dark bottle in order to prevent degradation from light. This solution was mixed with aqueous peel extract in 9:1 ratio and kept for overnight till the color changes to dark brown indicating the formation of silver nanoparticles which were harvested after centrifuging the solution and re-dispersing the precipitate in milli Q water to get purified particles. They were oven dried and used for further characterization and bioactivity study.

2.5 Characterization of silver nanoparticles

Firstly, UV-VIS Spectrophotometric analysis was done to confirm AgNPs fabrication which was monitored at different time interval by diluting a small aliquot of the sample with milli Q water. Silver nanoparticle solution was prepared and used for making a thin film on a glass slide for characterization through Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) for studying their size, shape and surface morphology. Dynamic Light Scattering (DLS) analysis was done to measure the size distribution in a colloidal solution.

2.6 Collection and Rearing of mosquito larvae

Third instar *A. aegypti* larvae were collected from various breeding localities of Agra through standard dipping procedures and brought to the laboratory. These larvae were maintained in dechlorinated tap water and provided with standard fish food under laboratory conditions.

2.7 Mosquito Larvicidal Bioassay

Larvicidal bioassay was carried out according to the standard procedure of World Health Organization [11] to check the effectiveness of synthesized AgNPs. Bioassay was tested on 20 mosquito larvae with 200ml of distilled water in beakers for III instar larvae at different concentrations of AgNPs. Besides this, permethrin was run in parallel with an experimental setup as a positive while dechlorinated tap water as a negative control. Five replicates for each concentration were tested. Mortality was observed after the exposure of test concentrations and expressed as median lethal concentrations at which 50% (LC_{50}) and 90% (LC_{90}) mortality occur, which was calculated by Probit analysis [12].

2.8 Nitric Oxide scavenging assay

NO scavenging assay was performed by using Kang *et al*.[13] protocol. For this assay, 1.0 ml of 5.0 mM sodium nitroprusside mixed with 1.0 ml of different concentration of Banana (10-100ppm) AgNPs. This solution mixture was incubated for 60 min at room temperature and an equal amount of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% napthylethylene diamine dihydrochloride) was added to it. Absorbance was measured at 546 nm with the help of UV-VIS spectrophotometer.

2.9 Statistical analysis

Probit analysis is expressed in terms of percent inhibition with respect to control at the median lethal concentration (LC_{50}) and LC_{90} for all instar larvae. All the tests were performed in five replicates and data expressed are as mean ± SD. The mortality below 20% was corrected by Abott's formula as follows:

Abott's formula = T-C/100 – C x 100

T =% Mortality of test organism,
C =% Mortality of control

3. Results and Discussion

Today, societies greatly rely on a sustainable model for food and water which can only be derived from natural resources. This model can only be made effective with the inclusion of waste management of all types such as bio-waste, mineral waste and day to day household waste. The sustainable model includes the cost of collection, transport and recycling of all these wastes to prevent mistreatment which may lead to hazardous diseases.

In the present study, we synthesized of stable silver nanoparticles with the bioreduction method using biowaste from Banana peel. UV-VIS spectral scanning was done in the wavelength range from 200-700nm to validate the fabrication of silver nanoparticles. The color change of silver nitrate solution was observed after different time intervals viz. 1 hr, 2 hr, 4 hr and 24 hr and spectra were recorded with a sharp peak observed at 426 nm wavelength (Figure 1). It is evident from the color change that Ag (I) has been converted into Ag (0) as the color intensity increased with reaction time.

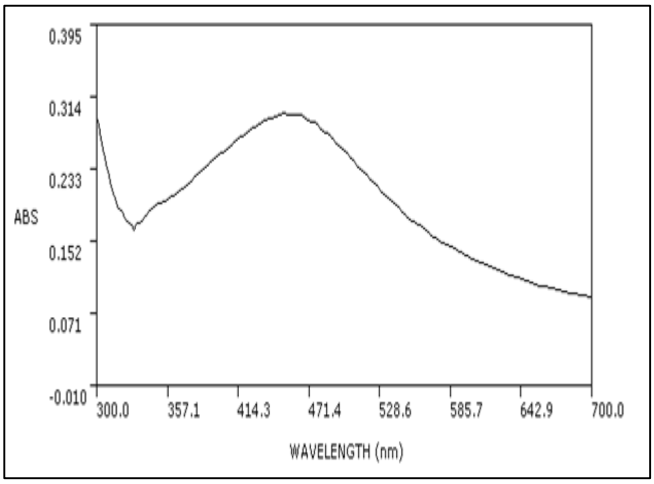

Fig 1: UV-VIS spectral analysis in the wavelength range from 300-700nm after various time intervals with sharp peak after 24 hrs at 426 nm

Scanning electron microscopy was done to observe the surface morphology of silver nanoparticles. These nanoparticles are spherical in shape (Figure 2a). Transmission electron microscopy (TEM) was done to calculate the size of silver nanoparticles synthesized from Banana peel extract *i.e.* 31nm. Figure 2b clearly demonstrate nearly spherical shape of AgNPs and slightly aggregated may be due to biomolecules of banana peel extract. Further, their size ranged between 20-50 nm which is comparable with the results of other researchers who synthesized AgNPs using plant extract by Salunkhe *et al.* [14] and Veerakumar *et al.* [15]. Dynamic Light Scattering (DLS) analysis was conducted to calculate average particle size as 81.74nm in a colloidal solution (Fig. 3).

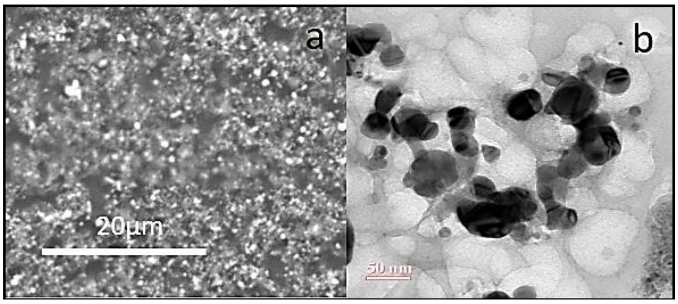

Fig 2:a) Scanning electron micrograph depicting surface topography of AgNPs; **b)** Transmission electron micrograph was depicting size and shape of AgNPs.

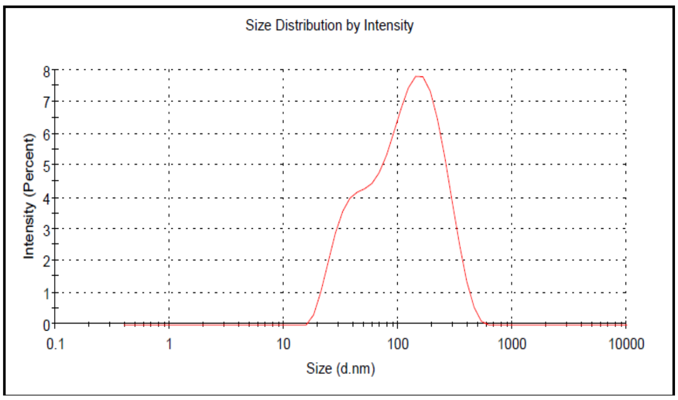

Fig 3: Size Distribution of Banana AgNPs through DLS.

After a successful synthesis of AgNPs from biowaste, its utility was tested for managing the mosquito population as a control measure to suppress disease transmission. Hence, laboratory experiments were conducted using standard WHO [11] protocols that depicted promising control bioactivity against III instar larval stages. Nearly 5- 15% mortality was observed in various control groups over the course of the experiment. The size of larvae strongly influenced the pattern of mortality and it increased as the test concentrations increased.

Figure 4 demonstrates a relationship between probit of kill and log concentration for III instar larvae as depicted through probit regression line. These were used to calculate median lethal concentrations as LC_{50} and LC_{90} values for III instar larval stages along with lower fiducial limits (LFL) and upper fiducial limits (UFL) and presented in (Table 1). LC_{50} was calculated as 25.15, 12.01 and 10.69 ppm for III instar larvae after 24, 48 and 72 hours. Similarly, LC_{90} values are also presented in Table 1 proving these nanoparticles effective in suppression of mosquito population in water bodies. Recently, many researches have been conducted on the larvicidal potential of AgNPs synthesized using plant parts with major focus on the leaves and it was observed that biosynthesized AgNPs were effective in controlling mosquito population [16-20].

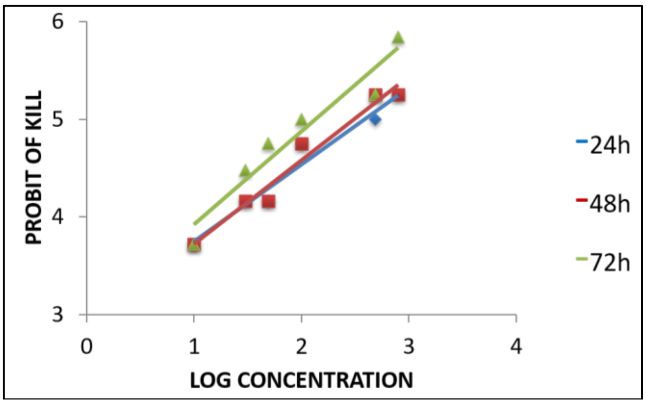

Fig 4: Relationship between probit of kill and log concentration of silver nanoparticles synthesized using Banana peel extract against III instar larvae of *Aedes aegypti* following 24h, 48h and 72h exposure

Table 1: Bioactivity of silver nanoparticles synthesized using Banana peel extract against *Aedes aegypti* III instar larvae

Hours	LC_{50} (ppm) (LFL-UFL)	LC_{90} (ppm) (LFL-UFL)	Equation	Chi square
24h	25.15 (21.69-30.05)	88.83 (61.99-173.33)	Y=2.338X+1.725	5.01
48h	12.01 (10.2-13.78)	63.8 (47.2-101.8)	Y=1.766X+3.09	5.54
72h	10.69 (8.96-12.25)	29.5 (22.54-35.77)	Y=2.908X+2.007	8.93

LFL- Lower fiducial limit; UFL- Upper fiducial limit

Nitric oxide (NO) considered as a free radical and reacts with reactive oxygen species which generate free radical in the form of reactive nitrogen species. NO was effective enough to fight against oxidative stress in a physiological system. NO scavenging assay was conducted to evaluate the antioxidant activity of Banana AgNPs as 71.73±1.26 maximum free radical scavenging activity was obtained at 1 ppm concentration. The lowest free radical scavenging activity was calculated as 56.99±7.48 at 100 ppm concentration as compared to standard Ascorbic acid. Figure 5 depicted the percent free radical scavenging activity against the concentration of Banana AgNPs. The standard Ascorbic acid scavenging activity was calculated as 80.21%. Detailed NO scavenging activity is listed in Table 2. Few researchers reported that NO scavenging activity of silver nanoparticles was less than the standard ascorbic acid and this was due to the phytopotentials of plant extract that stabilized the AgNPs and enhanced the bioactivity of green synthesized silver nanoparticles [21, 22].

Table 2: Nitric oxide (NO) scavenging activity of silver nanoparticles synthesized using Banana peel extract against *Aedes aegypti*

Concentration (PPM)	Nitric oxide (NO) Activity (%)
1	71.732
10	70.516
25	65.252
50	65.64
100	56.992
Standard	80.213

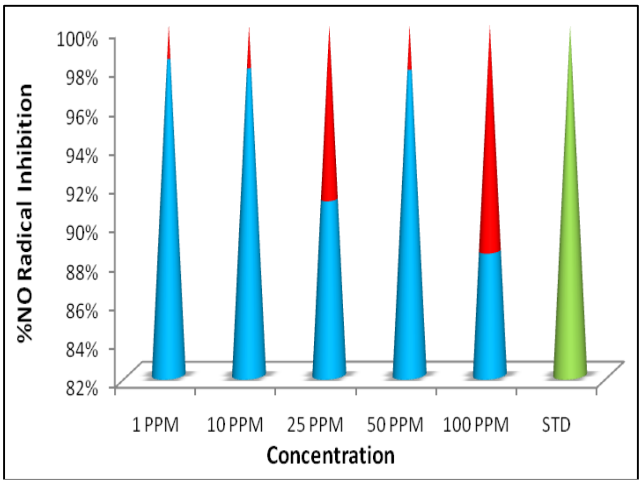

Fig 5: Nitric oxide (NO) scavenging activity of silver nanoparticles synthesized using Banana peel extract against *Aedes aegypti* (Red peaks indicate standard deviation)

Nowadays, garbage or solid waste has been an issue and we have fabricated silver nanoparticles for managing one such biowaste *i.e.* Banana peels which are extensively created. These peels are a potential source of natural antioxidants, which stabilize and reduce oxidative stress in cells. Green synthesized silver nanoparticles were considered safe due to their biodegradable nature as they were stabilized by the phytopotentials of Banana peel extract. Moreover, antioxidative properties of green synthesized AgNPs were helpful in enhancing their bioactivity thus killing the disease vectors. This green nanobiotechnology approach holds tremendous potential for not only managing rich biowaste but also their applications in managing the disease vectors.

4. Conclusions

In the current study, biowaste management strategies have been adopted for reducing environmental impacts and offers economic opportunities using a nanotechnology approach for biofabrication of silver nanoparticles using Banana peel extract. Firstly, biofabricated AgNPs was confirmed through UV-VIS spectrophotometer ranges from 300-700nm, after that SEM, TEM and DLS analysis was conducted for depicting their size and shape *i.e.* stable, spherical and crystalline ranges from 20-50nm. Banana peel extract showed remarkable toxic effects on III instar larvae after 24 hrs, 48 hrs and 72 hrs of exposure. NO scavenging assay showed that biofaricated Banana AgNPs were effective enough to fight against the oxidative stress in a system. This study highlights the potential biowaste management strategy by employing a nanotechnology approach for the enhancement of larvicidal activity of AgNPs that serve as a better alternative for dengue vector management.

5. Acknowledgments

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