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Biosynthesised silver nanoparticle with *Rhizophora annamalayana* mangrove leaf aqueous extract and its larvicidal activity: An *in-vitro* study

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Abstract
Plant extracts have been used as both reducing and capping agents, which has helped make the production of silver nanoparticles (AgNPs) more eco-friendly. Following a 30-minute treatment with aqueous leaf extracts, a change in color was evident, indicating the formation of silver nanoparticles (AgNPs). Moreover, the confirmation was achieved by analyzing data acquired from a range of analytical techniques, such as the UV-visible spectrum, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning Electron Microscopy (SEM). The larvicidal activity of these silver nanoparticles (AgNPs) was evaluated against the third instar and fourth larvae of *Aedes aegypti* in the present study. The results indicated that the larvae displayed a reaction that differed depending on the dosage, with AgNPs at 20 mL demonstrating the most notable effect on mortality rates. More precisely, when the concentration was 90 µg/mL, the mortality rate was 84% for 3rd instar larvae and 87.33% for 4th instar larvae. In contrast, the concentration of 12.5 µg/mL resulted in the lowest amount of activity, specifically 12.67%, on 3rd instar larvae. This phenomenon can be attributed to the likelihood of the radicals forming chelation bonds on the extensive surface area of the nanoparticles. The study demonstrates the potential use of *R. annamalayana*-mediated AgNPs as highly effective larvicidal agents for the development of innovative nanoparticle-based biological treatments.

Keywords: Silver nanoparticles, mangrove extract, larvicidal activity, *Rhizophora annamalayana*

1. Introduction

Vector-borne diseases are a major cause of death and illness among people living in tropical and subtropical areas. Studies have shown that vector-borne diseases are currently affecting more than one billion people [1]. The Anopheles, *Aedes* and *Culex* genera are responsible for deadly diseases like Chikungunya, Dengue, Malaria, Zika, Yellow Fever, West Nile, Rift Valley Fever, Japanese Encephalitis, Dog Heartworm, Murray Valley Encephalitis, and Western Equine Encephalitis. Mosquito-borne diseases, transmitted to people by vectors, are highly prevalent in numerous countries worldwide. Annually, a substantial proportion of individuals worldwide, amounting to billions, receive a diagnosis of one of these illnesses. India harbors a significant share of the global affected population, with approximately 40 million individuals being plagued by one of these ailments. The transmission of the most prevalent endemic diseases in Southeast Asia, the Pacific Islands, Africa, America, and India is primarily facilitated by the vector *Aedes aegypti*. The *Aedes aegypti* mosquito is widely recognized as the principal carrier of the dengue virus in the Southeast Asian region [2, 3]. The increasing number of documented cases has led to the evolution of dengue fever into a complex public health issue. The prevalence of dengue fever in India has been on the rise, as evidenced by annual data showing a comparable increase in mortality rates. In 2017, a substantial population of 31,117 individuals across many states and union territories were affected by the consequences of dengue. In addition, there were 48 recorded deaths caused by this illness in a relatively short period of time. Kerala has the highest prevalence of dengue cases among the several states in India, with a total of 14,806 cases, while Tamil Nadu closely trails with 5,968 cases [4].

The primary and exclusive objective of tackling the aforementioned disease epidemics is the implementation of vector control methods. The inclusion of natural products in vector control strategies is motivated by their plentiful supply of bioactive compounds, their ability to specifically target particular vectors, and their ability to break down naturally. The use of plant-derived insecticides has gained considerable attention due to their alleged ecological compatibility and improved safety in comparison to synthetic pesticides and medications. Plant-based insecticides provide a viable substitute for synthetic pesticides in the field of pest management. The reason for this claim lies in the fact that plant-based insecticides provide significantly less risk to human health and the natural ecosystem in comparison to synthetic alternatives [5].

Nanoparticles have been a major field in pest management, serving as a new method in recent years. Nanoparticles are highly valuable in diverse biological and agricultural applications, including protecting crops, enhancing plant growth, detecting plant diseases, optimizing nutrient uptake in plants, and resolving environmental issues. Nevertheless, it is important to acknowledge that the application of nanoparticles in these fields is currently in its nascent phase of advancement [6]. Research on ecologically sustainable approaches to nanoparticle synthesis is a prominent topic in the field of nanotechnology. Recently, there has been a growing use of biosynthetic techniques involving the utilization of biological microbes, fungi, or plant extracts. These strategies have gained popularity as a convenient and practical substitute for more intricate chemical synthetic methods in the manufacturing of nanomaterials [7, 8]. The utilization of green synthesis methodologies in the creation of nanoparticles offers several advantages in comparison to traditional chemical methods while also ensuring environmental sustainability. It has been demonstrated that this strategy is effective in lowering the number of mosquito vectors in an area [9]. Mangrove plants possess a diverse range of phytochemicals that are crucial for the production of nanoparticles. This property renders them a potentially advantageous alternative resource for the development of strong mosquitocidal drugs aimed at combating mosquito vectors. Mangrove leaf extracts have advantageous properties including stimulant, antispasmodic, and insecticidal qualities [10, 11]. Despite the available literature on the larvicidal properties demonstrated by specific mangrove species against different mosquito vectors, there is a lack of information about the efficacy of *R. annamalayana* species in the control of dengue vectors. In the present study, the objective was to synthesize, characterize, and examine the larvicidal properties of silver nanoparticles (AgNPs) derived from the leaves of *R. annamalayana*, a distinct species of natural hybrid mangrove plants. The target of the investigation was the efficacy of the synthesized AgNPs against 3rd and 4th instar larvae of *Aedes aegypti*.

2. Materials and Methods

2.1. Collection of plant materials and Preparation of Extract

Fresh leaves of *R. annamalayana* were obtained from the Pichavaram Mangrove forest area in the Cuddalore district of Tamil Nadu, India, ensuring that no harm was caused to the tree's stem. (Fig.1). To create the aqueous leaf extract, 10 g of finely chopped and powdered leaves were used. The solid substance was combined with 200 ml of sterile milliQ water in a 500-milliliter Erlenmeyer flask and thereafter subjected to boiling at a temperature of 60 °C for duration of 5 minutes. Following the boiling process, the extract underwent filtration, and subsequently, the resulting filtrate was stored at a temperature of 4 °C for subsequent utilization.

Fig 1: *R. annamalayana*

2.2. Solvent extracts derived from *R. annamalayana* leaves

A substantial quantity of plant leaves was collected, washed under a continuous flow of water, subjected to forced air in an oven at a temperature of 60 degrees Celsius for a duration of three hours, and subsequently pulverized to achieve a granulometry of five millimeters. The samples were subsequently placed in plastic containers and stored in the refrigerator at a temperature of approximately 4 °C until the commencement of the bioactive chemical extraction procedure. The solid-liquid extraction experiments were conducted using water as the selected solvent in an orbital shaker (Remi Mini Rotary Shaker, RS-12R, India). The extraction process was conducted in 500-ml Erlenmeyer flasks, following specific operating parameters: a temperature of 30 ºC, a stirring speed of 150 rpm, an extraction time of 18 hours, and a ratio of 30 g of *R. annamalayana* leaves per liter of water. The resulting extract underwent centrifugation, and the solid particles were isolated by passing them through Whatman No. 1 filter paper. The remaining liquid was then filtered under vacuum. A total of 350 ml of extract was collected and thereafter stored in topaz-colored glass jars to mitigate the impact of photo-oxidation on its constituents [12].

2.3. Mosquito larval Rearing

The *Aedes aegypti* larvae were obtained from the drainages situated in a residential locality in Chidambaram, Tamil Nadu, India. The larvae were placed in plastic enamel trays and provided with yeast powder as their source of nourishment, all while being kept within the controlled environment of the laboratory. The experiments were carried out under controlled conditions, with a temperature range of 27±2 °C and a relative humidity range of 75–85%. The trials also followed a light-and-dark cycle of 14 hours of light followed by 10 hours of darkness.

2.4. The Larvicidal bioassay

The larvicidal bioassay was done on *Aedes aegypti* larvae in their early third and fourth instars using different solvents and extracts from the current mangrove species. The procedures employed in this study were consistent with those previously employed by other researchers (13, 14). The *Aedes aegypti* larvae were obtained from the nearby vicinity with the aid of entomology researchers (Fig. 2). Subsequently, the third and fourth instar larvae were carefully separated and transferred to the laboratory for the purpose of the current investigation. The *Aedes aegypti* larvae were kept in controlled conditions within an insectariums with a temperature range of 28±2 °C and a relative humidity of 80±5%. Their daily diet consisted of a mixture of 4 g of Yale Fortune Cabin Sweetened Biscuit and hydrolyzed yeast at a ratio of 3:1. The bioassays utilized third-instar larvae that were in a healthy state. The approach provided by the World Health Organization (2005) was employed to conduct the larvicidal activity. In the bioassay, a total of 50 larvae in good health were distributed into separate beakers. These beakers contained manufactured AgNPs at volumes of 5 mL, 10 mL, and 20 mL, respectively. The AgNPs were present in varying concentrations (90, 45, 25, and 12.5 µg/mL) and were dissolved in dimethyl sulfoxide (DMSO). Control groups were also included, consisting of larvae treated with DMSO alone. The experiment was conducted with three replications for each test. The mortality of the larvae was documented 48 hours following the treatment. Probit analysis was employed to ascertain the larval mortality percentage as well as establish the median lethal concentration (LC_{50}) and lethal concentration (LC_{90}) [15].

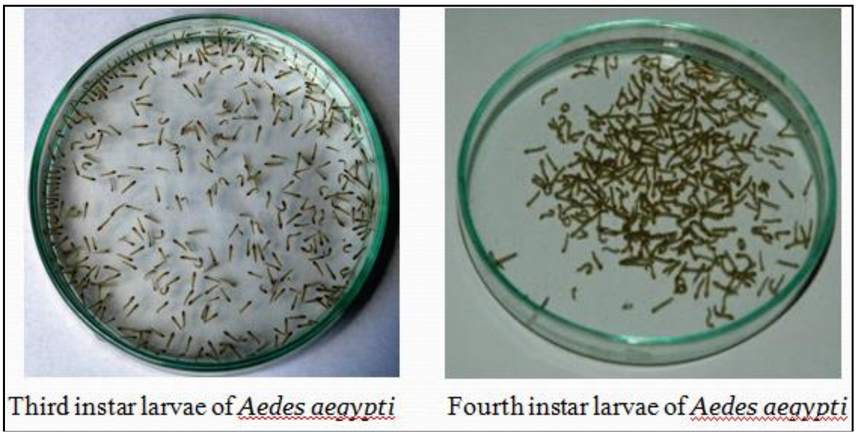

Fig 2: 3rd and 4th larvae of *Aedes aegypti*

3. Results and Discussions

3.1. Confirmation and Characterization of AgNPs

Photocatalysis was used to produce AgNPs by reducing silver ions in a reaction mixture containing $AgNO_3$ and an aqueous extract of *R. annamalayana* leaves. After being exposed to sunlight for 30 minutes, there was a noticeable change in color from a light yellowish hue to a brown shade. The validation of AgNP synthesis was accomplished by employing UV-Vis spectroscopy in aqueous solutions. The spectral scan obtained after a 30-minute reaction under sunlight showed a distinct peak at 420 nm for the $AgNO_3$ solutions. The presence of this peak indicates the formation of AgNPs, and the observed color is a result of the activation of surface plasmon resonance (SPR) in the AgNPs. However, the lack of a clear peak at 420 nm in the reaction performed in the absence of light suggests that the fast production of AgNPs, aided by the phytochemicals present in *R. annamalayana* extract, requires the activation of light and an optimal concentration of AgNO3 solution [16, 17]. Various analytical approaches were used to study and verify the process of biogenesis of silver nanoparticles (AgNPs). The confirmation of the nanostructure of the synthesized extract was achieved by analyzing data obtained from several analytical techniques, including the UV-visible spectrum, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), energy-dispersive X-ray spectroscopy (EDX), scanning electron microscopy (SEM), and high-resolution transmission electron microscopy (HR-TEM). The larvicidal properties of this extract were additionally evaluated for the purpose of the present investigation.

3.2. The larvicidal activity of silver nanoparticles

Tables 1 and 2 display the mortality percentages of larvae at various concentrations that were subjected to testing and subsequently determined, alongside the LC_{50} and LC_{90} values, following a 48-hour period of exposure. The larvicidal efficacy of silver nanoparticles (AgNPs) at concentrations of 90, 45, 25, and 12.5 µg/mL, along with the aqueous extract derived from *R. annamalayana* leaves, was evaluated against third instar and fourth instar larvae of *Aedes aegypti*.

Table 1: The larvicidal efficacy of AgNPs and *R. annamalayana* leaf extract and *R. annamalayana* leaf extract themselves against third instar larvae of *Aedes aegypti*

Concentration (µg/mL)	Mortality (%) after 48 h			
	AgNPs @ 5 mL	AgNPs @ 10 mL	AgNPs @ 20 mL	*R. annamalayana* leaf extract (ml/L)
90	39.33±4.16	58.00±3.46	84.00±3.46	34±2.00
45	22.00±2.00	34.00±2.00	42.00±2.00	22.67±3.06
25	14.67±3.06	23.33±3.06	23.33±2.31	15.33±1.15
12.5	4.67±1.15	6.00±2.00	12.67±1.15	8.67±1.15
Control				
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
LC_{50} & LC_{90} experiment				
LC_{50} (µg/mL)	104.67±1.53	91.00±2.00	67.00±2.65	210.33±2.52
LC_{90} (µg/mL)	197.67±2.08	135.67±3.79	120.33±3.06	297.33±2.52

Table 2: The larvicidal efficacy of AgNPs and *R. annamalayana* leaf extract themselves against fourth instar larvae of *Aedes aegypti*

Concentration (µg/mL)	Mortality (%) after 48 h			
	AgNPs @ 5 mL	**AgNPs @ 10 mL**	**AgNPs @ 20 mL**	***R. annamalayana* leaf extract (ml/L)**
90	32.00±3.46	72.00±3.46	87.33±3.06	26±3.46
45	24.00±2.00	43.33±3.06	50.67±3.06	21.33±1.15
25	15.33±1.15	32.00±2.00	39.33±3.06	13.33±3.06
12.5	11.33±1.15	26.67±3.06	24.00±2.00	6.00±0.00
Control				
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
LC_{50} & LC_{90} experiment				
LC_{50} (µg/mL)	113.67±3.21	96.67±1.53	83.00±2.65	252.67±3.06
LC_{90} (µg/mL)	201.33±5.03	204.00±3.61	137.33±2.52	344.00±2.65

The larvicidal activity results of the silver nanoparticles (AgNPs) demonstrated a reaction that was dependent on the dosage administered to the larvae. The larvae treated with AgNPs at concentrations of 20 mL exhibited the highest mortality rates. Specifically, the third instar larvae showed a mortality rate of 84%, while the fourth instar larvae displayed a mortality rate of 87.33% at a dose of 90 µg/mL. The LC_{50} and LC_{90} values of the mangrove extract now under investigation were assessed in third-instar larvae and found to be 210.33 and 297.33 µg/mL, respectively.

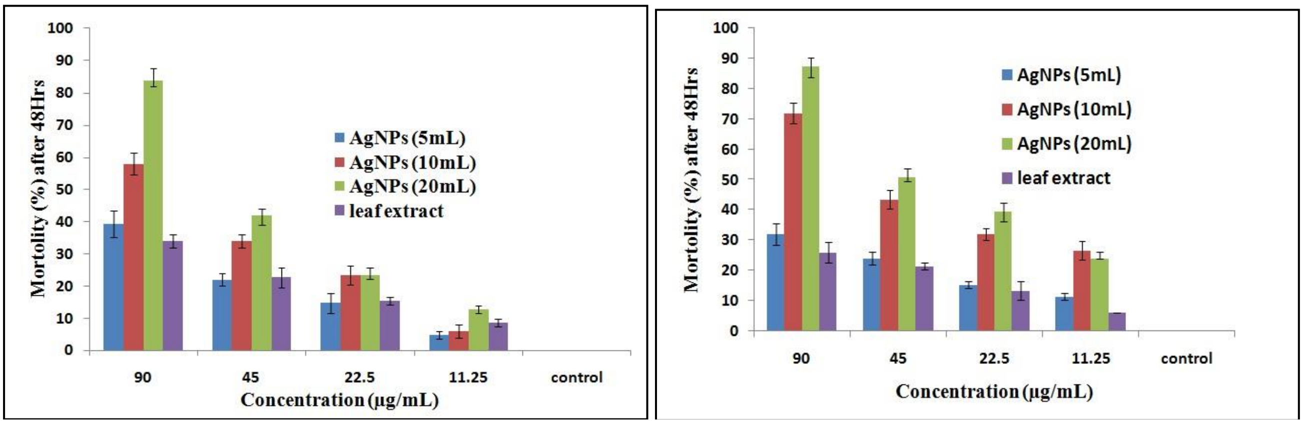

Fig 3: The effectiveness of AgNPs and *R. annamalayana* leaf extract in killing 3rd (left) and 4th (right) stage larvae of *Aedes aegypti*

The development of pesticides that possess both non-toxic and efficacious properties is of utmost significance for worldwide public health. This study investigates the possible utilization of *R. annamalayana*, a coastal plant recognized as a natural hybrid species of mangrove, as a viable source of environmentally friendly Nano insecticides and growth inhibitors specifically tailored to combat *Aedes aegypti*. The larvicidal effectiveness of silver nanoparticles (AgNPs) synthesized from *Avicennia marina* leaf extracts was significant against *Aedes aegypti* and *Aedes stephensi*. The peak degree of action was reported at AgNPs concentrations of 5 and 10 mg/l, specifically targeting fourth-instar larvae. In the study, it was demonstrated that eradicating Aedes aegypti larvae using silver nanoparticles, which were extracted from the leaves of A. marina and a few other plants, was extraordinarily effective [16-19], which aligns with the present study and establishes a distinct correlation. Functionalized nanoparticles with capping agents exhibit heightened surface reactivity, rendering them highly beneficial for a diverse array of applications. The larvicidal effects of silver nanoparticles (AgNPs) can be related to their capacity to infiltrate and impair cellular metabolism by binding to DNA and enzymes [20].

The observed larval mortality can be linked to the morphological damage of different organs in the larvae, which is caused by the buildup of nanoparticles. The buildup of this substance probably resulted in interactions with proteins in the cells, which hindered the enzymatic activities in the larvae. The proposal posits that nanoparticles have an increased surface area, which allows them to be taken up by biological membranes. The process of internalization has the potential to weaken the integrity of the membrane and cause the release of silver, which could be poisonous to larvae or pathogenic organisms [21, 22]. The findings indicate that higher concentrations of the leaf extract resulted in elevated mortality rates in relation to the synthesis of AgNPs. The observed mortality, which varies depending on the dosage administered, is consistent with findings reported in previous research [23]. The death of *Aedes aegypti* larvae was detected when they were exposed to AgNPs, indicating that AgNPs have the potential to be used as a larvicidal agent. Prior research has shown that silver nanoparticles, produced by environmentally friendly techniques utilizing the leaf extract of *Excoecaria agallocha*, were examined at different concentrations (ranging from 2.0 to 14 mg/l) to assess their impact on the third and fourth instar larvae of *Aedes aegypti*. The researchers discovered that the third instar larvae of *A. aegypti* had the highest death rate. After being exposed for 24 hours, the LC50 values for these larvae were determined to be 4.65 and 6.10 mg/L, respectively. The present study's findings further show that larvicidal activity is accomplished at a relatively low concentration of AgNPs on mosquito larvae (24). There are many publications [25, 26] that discuss the significant potential of AgNPs as Nano insecticides from a wider perspective. The present study's results may be attributed to the presence of a marine extract containing a potent compound that has larvicidal properties against mosquitoes, as confirmed by prior research.

4. Conclusion

The present study investigates the use of leaf extract from *R. annamalayana* for the production of silver nanoparticles (AgNPs) as a promising biolarvicidal agent against the mosquito vector *A. aegypti*. The study examined the impact of silver nanoparticles (AgNPs) produced by mangrove plants on populations of vector mosquitoes. The findings demonstrated that the eco-friendly production of silver nanoparticles (AgNPs) had a substantial and swift impact on the population of vector mosquitoes. Hence, it was deduced that employing green synthesized AgNPs, which are environmentally friendly and sustainable, could be a more efficient approach for vector control and management. This technique yielded small-sized nanoparticles with spherical shapes and a crystalline structure. The study showcased the robust larvicidal efficacy of the produced silver nanoparticles without any adverse effects on non-target aquatic insects. Hence, it is crucial to consider the biocompatibility of these silver nanoparticles as being non-toxic. Considering this, they have the potential to be employed for pest control and administering drugs to patients affected by infectious illnesses in the future.

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