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In vitro investigation on the Larvicidal activity of the mosquito *Aedes aegypti* with biosynthesised silver nanoparticles from the seaweed extract of *Kappaphycus alverazii*

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Abstract
Mosquitoes are the main vectors of diseases like chikungunya, dengue, and malaria. Neurotoxic pesticides have negative effects on humans and ecosystems. Nanotechnology, using plant extract nanoparticles like AgNPs, is being explored for pest eradication. Seaweed species, abundant in marine environments, have similar insecticidal qualities to land plants and fungi. The present study examines silver nanoparticles from *Kappaphycus alverazii* and AgNPs produced through biosynthesis. *K. alverazii* seaweed was collected from Thondi, South India, pulverized, and boiled into an extract. The study characterizes *K. alverazii* silver nanoparticles using various techniques, including UV-Vis spectroscopy, FTIR, SEM, EDAX, and XRD. The researchers used photocatalysis to synthesize AgNPs by reducing silver ions in a reaction mixture with $AgNO_3$ and an aqueous seaweed extract. The color change was observed after 30 minutes of exposure to sunlight, indicating the formation of AgNPs. These nanoparticles were tested on *Ae. aegypti* larvae, assessing their larvicidal activity and mortality rates. The study found that the larvicidal properties of *K. alverazii* AgNPs exhibited varying effects on larvae depending on their exposure level. The mortality rate varied from 85.33% for third instar larvae to 90.67% for fourth instar larvae. Control was maintained throughout the study, and no larval mortality was observed. As a consequence of this, the current study is to investigate the larvicidal activity of *K. alverazii* extract and its AgNPs on larvae of *Ae. aegypti* in their third and fourth instars.

Keywords: *Kappaphycus alverazii*, seaweed, silver nanoparticles, larvicidal

Introduction

Mosquitoes carrying illnesses and parasites spread many diseases, including chikungunya, dengue, lymphatic filariasis, malaria, yellow fever, the Zika virus, and Japanese encephalitis. These diseases are a major problem around the world. [1]. Mosquitoes are the primary vectors responsible for carrying the disease. However, not all mosquitoes have the ability to spread diseases, especially malaria. Specifically, the spread of illness is associated with mosquitoes belonging to the Anopheles genus. There are over 430 species of Anopheles mosquitoes, but only a certain subset of them have the ability to efficiently transmit malaria. Among these total members of Anophelus, *Aedes aegypti* and *Aedes albopictus* are responsible for transmitting diseases such as dengue fever, chikungunya, zika fever, and yellow fever [2]. Neurotoxic pesticides, such as neonicotinoids, pyrethroids, organophosphates, and carbamates, have been extensively employed during the last century to specifically target adult mosquitoes for control purposes. Chemical insecticides that specifically target the adult stage of the mosquito's life cycle have been the main approach to mosquito control in recent decades [3, 4, 5, 6]. These chemical insecticides have adverse effects on humans and can disrupt the ecosystem by harming non-target creatures, including beneficial insects. Moreover, the occurrence of insect resistance, contamination of natural resources, and consequent disruption of the food chain result in a decrease in biodiversity [7]. The continuous utilization of these insecticides has resulted in the emergence of mosquito populations that possess resistance to these toxins. The persistent use of synthetic insecticides to control mosquito larvae has led to various issues, including the development of mosquito resistance and adverse effects on human health and the environment [8].

Nanotechnology is currently garnering significant attention due to its versatile applications in various domains, including electronics, medicine, and agriculture. Its potential for pest eradication also piques the interest of many people. Nanoparticles can be synthesized using conventional methods, including physical, chemical, and biological approaches. Currently, the utilization of plant extracts derived from roots, flowers, and stems to produce nanoparticles is regarded as a more secure alternative to conventional techniques. This approach is advantageous due to its cost-effectiveness, reduced environmental impact, and ability to be repeatedly employed [9]. According to previous studies, the utilization of nanoparticles to regulate mosquitoes has been examined, particularly focusing on mosquito vectors like *Aedes albopictus*, which are accountable for transmitting ailments such as dengue and Zika viruses. For instance, ZnONPs have demonstrated potential because of their eco-friendly characteristics and capacity to specifically target the mosquito vector. This particle induces damage to the mosquito's physiological processes and behaviors by compromising the integrity of the gut membrane. Nevertheless, the precise processes by which these specific nanoparticles impact mosquito larvae are now under investigation [10].

In addition to the mosquito-killing properties of nanoparticles, scientists are also investigating natural alternatives, particularly those derived from readily available seaweed species that are currently underutilized. Therefore, it is crucial to investigate the therapeutic components of seaweeds in order to assess their efficacy in mosquito eradication. Understanding the chemical components of seaweed is crucial for establishing the relationship between the structure and function of the compounds responsible for insecticidal properties [11]. Seaweeds, which are plentiful in the marine environment, are sustainable substances that can be classified into green, brown, and red groups according to their pigmentation. Seaweeds are currently being used in various businesses and medical fields because of their plentiful bioactive components, which are highly valued in biomedical research and the food industry [12]. Based on the scientific achievements mentioned above about mosquito elimination, several initial studies have suggested that seaweeds possess insecticidal qualities against mosquito larvae that are comparable to other bioinsecticides derived from land plants and fungi [13–15]. It has been reported that the brown algae *Sargassum wightii* is capable of synthesizing gold nanoparticles, and their antibacterial properties have been researched [16, 17]. In the present study, we have created and examined silver nanoparticles using an aqueous extract from *Kappaphycus alverazii*. In addition to this, the AgNPs that were produced through biosynthesis were evaluated for their ability to kill the instar larval stages of *Aedes aegypti*.

Materials and Methods

Seaweed collection and extraction process

K. alverazii, a type of seaweed, was collected from the coastal regions of Thondi, Tamil Nadu, located in South India. The gathered seaweed was meticulously rinsed with water to eliminate any sand and particles that were sticking to it, and then it was taken to the testing laboratory. The seaweed underwent four rounds of washing with distilled water and was subsequently dried in the shade. The dried components were pulverized into a fine powder using an electric blender to prepare the extract. The preparation of the extract involved combining 50 grams of pulverized seaweed with 500 ml of sterile distilled water in a 1000 ml Erlenmeyer flask. Subsequently, the substance was subjected to boiling on a heated surface for 30 minutes while being periodically stirred. The suspension was allowed to cool and then filtered using Whatman No. 1 filter paper. The resulting liquid was stored in a refrigerator until it was ready to be tested.

K, alverazii

Characterization of *K. alverazii* silver nanoparticles

The existence of artificially produced silver nanoparticles was examined at ambient temperature using a UV-Vis spectrophotometer (Shimadzu, Japan) within the wavelength range of 200 to 600 nm. In order to determine the potential functional groups present in the produced AgNPs solutions, the sample was analyzed using Fourier transform infrared (Perkin Elmer Spectrum 2000 FTIR spectrophotometer) spectroscopy within the range of 4000 to 1000 cm1. Portions of AgNPs were used for the Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDAX), and X-ray Diffraction (XRD) examinations. The morphology and form of the AgNPs were analyzed using a SEM equipped with energy-dispersive X-ray spectroscopy (EDAX). A total of 25 microliters of freeze-dried purified silver nanoparticles (AgNPs) were applied to a copper stub. The nanoparticles were then examined using an ultra-high-resolution scanning electron microscope (SEM) operating at 10 kilovolts (kV). X-ray diffraction spectroscopy was used to determine the phase variety and grain size of the produced nanoparticles. The acquired pictures were cross-referenced with the Joint Committee on Powder Diffraction Standards (JCPDS) library in order to validate the crystalline structure.

Mosquito sample collection and rearing

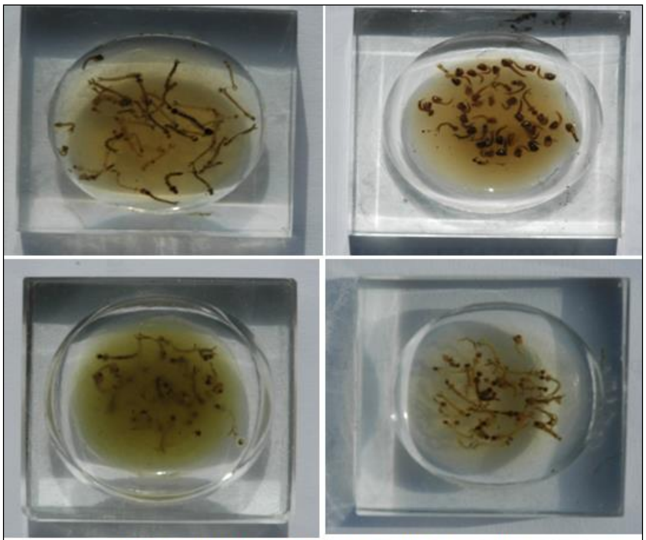
The larvae of *Ae. aegypti* were obtained from stagnant water in the vicinity and subsequently raised in a laboratory setting at the Department of Zoology, Thanthai Periyar Government Arts and Science College, Trichy, Tamil Nadu, India. The third instar larvae were identified from these mosquito larval samples and subsequently placed in plastic trays filled with tap water for the purpose of conducting the investigation. Further, they were then kept in the laboratory until they reached the early fourth instar stage, at which point they were used for the current investigation. All the larvae were fed with yeast powder. The larvae were raised in a controlled environment with a temperature range of 27±2°C and a relative humidity range of 75–85%. The trials were conducted following a light-and-dark cycle of 14 hours of light followed by 10 hours of darkness.

3rd instar larvae 4th instar larvae

Assessment of Larvicidal activity

The larvicidal activity of silver nanoparticles generated by *K. alverazii* was examined, following the guidelines provided by the World Health Organization [18]. During the bioassay, a total of 50 larvae in good health were evenly distributed into separate beakers, each containing different concentrations of the test extract for the current sample. The beakers held artificially produced *K. alverazii* AgNPs in quantities of 5 mL, 10 mL, and 20 mL, respectively. The quantities of *K. alverazii* AgNPs ranged from 90 µg/mL to 12.5 µg/mL and were dissolved in dimethyl sulfoxide

(DMSO). In addition, control groups were incorporated, consisting of larvae that were solely treated with DMSO. Each test was replicated three times during the experiment. The mortality of the larvae was assessed 48 hours after the treatment by transferring them to the watch bowl and recording their deaths. Probit analysis was used to determine the percentage of larval mortality and to establish the median lethal concentration (LC50) and lethal concentration (LC90).



Mortality watching of Larvicidal activity after 48Hrs

Results

The process of photocatalysis was employed to synthesize *K. alverazii* AgNPs by reducing silver ions in a reaction mixture including $AgNO_3$ and an aqueous extract derived from *K. alverazii*. Following a 30-minute exposure to sunshine, a discernible alteration in color occurred, transitioning from a pale yellowish tint to a brown hue. The verification of AgNP synthesis was achieved by utilizing UV-Vis spectroscopy in water-based solutions. The spectral scan acquired following a 30-minute reaction in sunlight revealed a prominent peak at 420 nm for the $AgNO_3$ solutions. The existence of this peak signifies the creation of AgNPs, and the perceived color is a consequence of the stimulation of surface plasmon resonance (SPR) in the AgNPs. Nevertheless, the absence of a distinct peak at 420 nm in the light-deprived reaction indicates that the rapid formation of AgNPs, facilitated by the phytochemicals found in *K. alverazii* extract, necessitates both light activation and an ideal concentration of $AgNO_3$ solution. The nanostructure of the synthesized extract was confirmed through the analysis of data obtained from various analytical techniques, such as the UV-visible spectrum, X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR). The characterizations were standardized for the current sample, and the results were also utilized for other applications such as antioxidant and antibacterial examinations of this extract.

The goal of this study was to find out how well K. alverazii AgNPs and an aqueous extract from K. alverazii can get rid of Ae. aegypti larvae in their third and fourth instars. The experiment included doses of 90, 45, 22.5, and 12.25 µg/mL. The median death rate, LC50, and LC90 levels were calculated after a duration of 48 hours. The findings were shown in Tables 1 and 2, revealing that the larvicidal properties of silver nanoparticles (AgNPs) exhibited varying effects on the larvae depending on the level of exposure they encountered. The larvae exposed to AgNPs concentrations of 20 mL/L or above exhibited the highest overall mortality rates. When the larvae were administered a dosage of 90 µg/mL, the mortality rate varied from 85.33% for the third instar larvae to 90.67% for the fourth instar larvae. The LC50 values for the third and fourth larvae were determined to be 88.33 and 78.33 µg/ml, respectively. The LC90 values of the *Ae. aegypti* larvae, which were exposed to *K. alverazii* AgNPs, were 188.33, 139.67, and 96.00 µg/mL, respectively, at the treatment concentrations of 5, 10, or 20 ml. After treatment with a concentration of 90 µg/ml, the measured mortality rate reached a maximum of 90.67%. The mortality rate of fourth-instar larvae was substantially higher in comparison to that of third-instar larvae. Control was successfully upheld throughout all stages of the studies, and no occurrences of larval mortality were observed in either the positive or negative control groups.

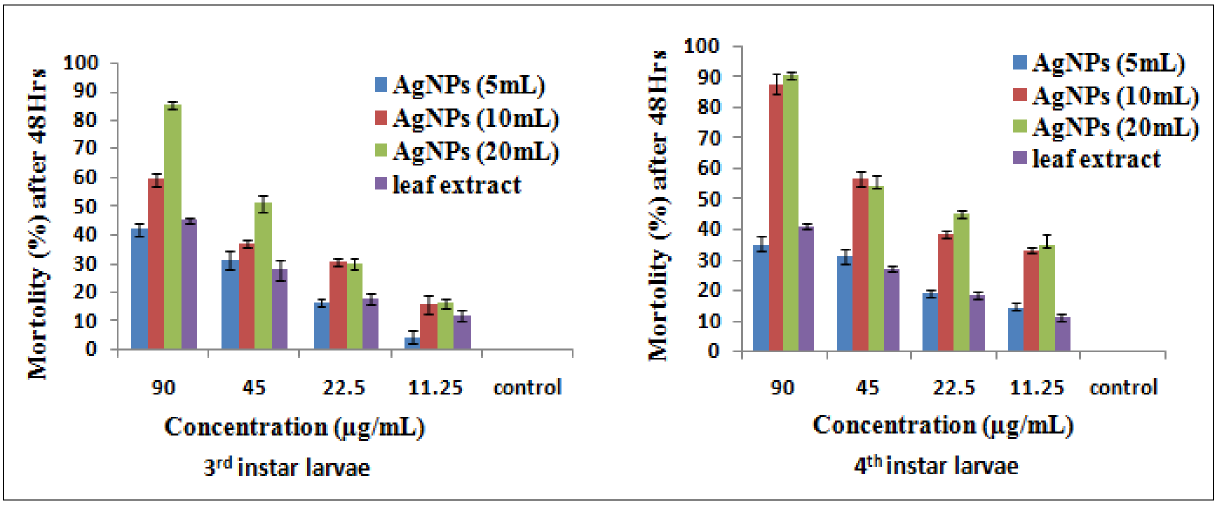
Table 1: The larvicidal efficacy of AgNPs of *K, alverazii* seaweed extract and *K, alverazii* extract themselves against third instar larvae of *Aedes aegypti.*

Concentration (µg/mL)	**Mortality (%) after 48 h**			
	AgNPs @ 5mL	**AgNPs @ 10mL**	**AgNPs @ 20mL**	***R. annamalayana* leaf extract (ml/L)**
90	42.00 ± 2.00	59.33 ± 2.31	85.33 ± 1.15	45.33 ± 1.15
45	31.33 ± 3.06	37.33 ± 2.00	51.33 ± 2.31	28.00 ± 3.46
22.5	16.67 ± 1.15	30.67 ± 1.15	30.00 ± 2.00	18.00 ± 2.00
11.25	4.67 ± 2.31	16.00 ± 3.46	16.67 ± 1.15	12.00 ± 2.00
Control				
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LC_{50} & LC_{90} experiments				
LC_{50} (µg/mL)	88.33 ± 1.53	69.33 ± 4.04	53.00 ± 2.65	155.00 ± 5.00
LC_{90} (µg/mL)	149.67 ± 4.51	125.00 ± 3.46	97.67 ± 2.52	212.67 ± 3.79

Table 2: The larvicidal efficacy of AgNPs of *K, alverazii* seaweed extract and *K, alverazii* extract themselves against fouth instar larvae of *Aedes aegypti.*

Concentration (µg/mL)	**Mortality (%) after 48 h**			
	AgNPs @ 5mL	**AgNPs @ 10mL**	**AgNPs @ 20mL**	***R. annamalayana* leaf extract (ml/L)**
90	35.33 ± 2.31	88.00 ± 3.46	90.67 ± 1.15	41.33 ± 1.15
45	31.33 ± 2.31	56.67 ± 2.31	54.67 ± 3.06	27.33 ± 1.15
22.5	19.33 ± 1.15	38.67 ± 1.15	45.33 ± 1.15	18.67 ± 3.06

11.25	14.67 ± 1.15	33.33 ± 1.15	35.33 ± 3.06	11.33 ± 1.15
Control				
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LC_{50} & LC_{90} experiments				
LC_{50} (μg/mL)	78.33 ± 2.89	77.67 ± 2.52	53.00 ± 2.65	194.00 ± 3.46
LC_{90} (μg/mL)	188.33 ± 2.89	139.67 ± 5.51	96.00 ± 2.65	232.67 ± 8.08



Discussion

The management of mosquitoes is a challenging, intricate, and costly endeavour. The mosquito trapping, fogging, insecticide treatment, and the use of natural predators are among the often employed techniques frequently adopted by public health agencies. The rising prevalence of dengue, encompassing both outbreaks and cases, despite the implementation of preventative methods, suggests that these techniques are either inadequate or ineffective. For example, research has shown that fogging is ineffective in halting the reproductive cycle of Aedes mosquitoes by eliminating the female mosquitoes, as the data does not indicate any notable distinction between the two groups [19]. The utilization of fogging and synthetic pesticides often exerts an adverse impact on both human health and the environment. Consequently, there is an urgent requirement for the investigation and advancement of pesticides that exhibit a reduced detrimental impact on the environment. The objective of this work was to assess the larvicidal efficacy of the *K. alverazii* extract and its AgNPs on third and fourth instar larvae of *Ae. aegypti*. The death effect observed in mosquito larvae as a result of AgNPs was attributed to the small size of the particles. This enables them to penetrate the insect's cuticle and enter individual cells, where they disrupt molting and other physiological processes [20]. The results of our investigation validated the prior research by illustrating that extracts derived from *Melia azederach* led to notable mortality rates within 24 to 48 hours of treatment on the larvae of the mosquito species *Culex quinquefasciatus* [21]. Administering nano-formulated seaweed metabolites has been shown to reduce mosquito populations during the larval and pupal stages [22, 23].

It is well believed that different types of nanoparticles have a harmful impact on arthropod tissues by causing oxidative stress [24, 25]. Further research indicates that the toxicity may be attributed to the infiltration of nanoparticles through the exoskeleton [26]. In line with our present research findings, Subramaniam *et al*. [27] documented that *Ae. aegypti* larvae experienced a mortality rate of 34% during the initial instar stage. However, the death rate increased to 89% when exposed to *A. vera* leaf extract at a concentration of 400 ppm. The silver nanoparticles (AgNPs) produced using environmentally friendly techniques shown significant effectiveness against *Cx. quinquefasciatus*, leading to a maximum mortality rate of 80% when applied at a concentration of 5 ppm. The death rates of *An. stephensi* and *Cx. tritaeniorhynchus* were comparatively lower. The toxicity of *Caulerpa scalpelliformis* in generating AgNP against *Cx. quinquefasciatus* was observed to be comparable to our findings [28]. Previous investigations suggest that the biomolecules, specifically phenols, terpenoids, and flavonoids, present in the chosen seaweed extract of K. alverazii are likely responsible for the observed mosquito-larvicidal effects when applied to the surface of AgNPs. This study represents the first report on the mosquito-larvicidal activities of AgNPs derived from *K. alverazii*.

Conclusion

The effective and long-term management of diseases transmitted by vectors is crucial to addressing a complex issue on a global scale. In a great number of countries, effective vector population management is an immediate necessity because of the prevalence of diseases that are transmitted by vectors. At the moment, a significant amount of focus is being placed on the development of biological control agents through the application of nano-biotechnological techniques. The creation of silver nanoparticles from the marine seaweed *K. alverazii* and the subsequent characterization of those nanoparticles are both presented in this research. These nanoparticles have properties that make them long-lasting, easily producible, harmless to the environment, and extremely effective even at relatively low concentrations. According to the findings of our research, *K. alverazii* AgNPs have larvicidal activities against the mosquito species *Ae. aegypti*. By putting this concept into action, there is a possibility that the use of synthetic pesticides, which have negative effects on the environment and on animals that were not the intended target, could be reduced. In addition, the creation of nano-bioformulations derived from seaweed will provide an additional alternative way of controlling vectors. We are optimistic that the findings of this first examination will point the way toward a fresh course of action in the field of seaweed nanobiotechnology research.

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