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Phytofabrication of silver nanoparticles using *Elephantopus scaber* and *Azadirachta indica* leaf extract and its effect on larval and pupal mortality of *Culex quinquefasciatus*

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ABSTRACT

Objective: To synthesize silver nanoparticles by using *Azadirachta indica* (*A. indica*) and *Elephantopus scaber* (*E. scaber*) leaf extract as reducing and stabilizing agent and to check the efficacy of silver nanoparticles towards mortality of mosquito larval and pupal stages of *Culex quinquefasciatus* (*Cx. quinquefasciatus*).
Methods: The silver nanoparticles were synthesized using *A. indica* and *E. scaber* leaf extract. The synthesized nanomaterials were characterized using UV-vis spectrophotometer, Fourier transform infrared spectroscopy, transmission electron microscopy and fluorescent microscopy. Finally, the synthesized nanoparticles were applied on fourth instar larval and pupal stages of *Cx. quinquefasciatus* larvae and pupae and mortality was counted for 48 h. Dead larval bodies were electron dispersive X-ray studied to see whether silver has entered into the body or not. Pupal hatching under the influence of these nanoparticles were also studied.
Results: Silver nanoparticles were synthesized from *A. indica* and *E. scaber* leaf which were stable. Mortality results highlighted that LC_{50} and LC_{90} of *A. indica* and *E. scaber* induced silver nanoparticle are 1.64, 4.32 mg/L and 1.43, 4.32 mg/L respectively during 48 h of incubation. EDX study of dead larval body showed a clear signature of silver in their body which suppose to be the main cause of their mortality. Pupal hatching is also hampered by the sublethal doses.
Conclusions: *A. indica* and *E. scaber* may be good source as reducing agent of stable silver nanoparticles. Green synthesized silver nanoparticles may be a good eco-friendly alternative to control mosquito larvae and pupae of *Cx. quinquefasciatus*.

1. Introduction

Mosquitoes are the disease causing vector that are responsible for transmitting malaria, filariasis and many other viral disease like dengue, Japanese encephalitis, yellow fever *etc.*[1]. Mainly three genuses of mosquitoes, namely, *Culex* sp., *Anopheles* sp. and *Aedes* sp. are widely distributed all over the world and cause millons of victims. *Culex quinquefasciatus* (*Cx. quinquefasciatus*) is the vector of filarial parasite *Wuchereria bancrofti*, responsible for human lymphatic filariasis. Moreover, *Culex* sp. transmits causative agents of avian malaria, St. Louis encephalitis, western equine encephalitis, West Nile fever *etc.*[2]. Although, death does not always occur due to filariasis, but it is the second leading cause of disability which imparts severe economic and social burden to the suffered person and his family. According to the report of National Vector Bourne Disease Control Programme[3], in 83 countries all over the world almost 120 million people are infected with this disease and it is predicted that 1.1 billion are at risk. About 40 million people are disabled by the infection of filarial parasite and 76 million people have hidden internal lymphatic infection with apparently normal appearance. According to World Health Organization report, among the infected people worldwide, 70% are from India, Indonesia, Bangladesh and Nigeria. *Cx. quinquefasciatus* is widely distributed all over the world, mainly in tropical and subtropical regions[4]. This mosquito prefers human dwellings for their breeding and they lay eggs in large partially polluted water bodies, rice fields, drains, tanks, ditches where high concentration of decomposed organic matter[5].

To successfully reduce the disease occurrence caused by the dipteral vectors, controlling the disease spreading vector mosquitoes, principally by applying insecticides at their breeding sites is the way of choice[6]. Application of synthetic mosquitocides, namely, dichloro diphenyl trichloroethane, dieldrin, organophosphorus, fenitrothion and propoxur is a practice for several decades, but this chemical insecticides has several demerits including resistance development[7], environmental degradation, bioaccumulation *etc.* Constant application of chemical controlling agents can cause harm to several organisms

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including human race which will ultimately lead to disruption of ecological balance. Prolonged exposure to chemicals may develop resistance mosquito strains. Therefore, a demand stems out for the synthesis of less harmful and more effective mosquito controlling agent. To control this deadly arthropod vector in a regulated way with minimum environmental harm nanomaterials may be an effective way.

Nanotechnology is now an emerging topic of scientific research. Nanomaterials are recently being utilized in various fields such as water treatment, medicine, catalysis, solar energy conversion *etc*. Nanosilver is good conductor, catalytic agent, chemically stable and have good antibacterial property and so it is now opted for several applications[8,9]. In china, nanosilver is used as anti-microbial agent in elevators of public place. Silver nanoparticles (AgNPs) are used for anti-microbial treatment in ointments, fabrics and even in surgery because of its anti-inflammatory, anti-angiogenic and anti-permeable properties[10]. However, for the last few years scientists have highlighted on the effective use of nanomaterials in the field of insect control[1,11,12]. AgNPs can be used as good mosquitocidal agent which is reported by many authors[5,13,14].

AgNPs can be synthesized by chemical process such as sodium borohydride reduction of monovalent silver ion to zero valent AgNPs[15]. UV irradiation induced polymetaacrylic acid cap for mosquito control by AgNPs were used by Sap-Iam *et al*.[16]. Biological synthesis of AgNP is more advanced than chemical and physical methods because green synthesis of AgNPs is cost effective and environment friendly. AgNPs can be synthesized biologically where fungi or bacteria are used for the reduction of silver ions to colloidal nano silver[17-19]. With the advent of utilization of micro organisms for the production of nanometals, use of plant biomolecules has also become a source of reducing agents. Many plants are utilized in bioremediation to purify heavy metals or to accumulate them. Hyper accumulator plants have some distinct mechanism to reduce it to lower oxidation state or chelation with bio ligands[20]. Those biomolecules of plants can be a possible agent to reduce low reduction potential metals to synthesize metal nanoparticles which is less time consuming, more cost effective, environment friendly and a single step process than physical and chemical methods[21]. Literature study reveals plant extracts are used to reduce silver ions as they contains several biomolecules like alkaloid, flavonoid, proteins, lectins, triterpenes, phenolics *etc*. These biomolecules are responsible for reduction of silver ions to nanoparticles and subsequent stabilization of nanoparticles by their capping effect to prevent the formed nanoparticles from being agglomerized. Some workers reported that proteins, carbohydrates and polyphenols are involved in AgNP synthesis[22] although the exact mechanism is still not clear. In this article, we report eco friendly and green synthesis of AgNPs using *Azadirachta indica* (*A. indica*) and *Elephantopus scaber* (*E. scaber*) leaf extracts as reducing and stabilizing agent, both of which has medicinal and aromatic property. Various bioactive molecules are present in these two leave extracts that can be utilized for reduction of metal solution to nanoparticles.

A. indica (common name: neem), typical for tropical and subtropical regions can be found in Indian subcontinent, Nepal, Bangladesh, Pakistan and Sri Lanka. Ayurvedic products made from neem is very popular in India for several years because it has medicinal properties such as antidiabetic, antimicrobial, antihelminthic, contraceptive, improve liver function. Various parts of neem contains diverse range of biochemicals. Govindachari *et al.*[23] reported antibacterial and antifungal property of quercetin and and ß-sitosterol, first phenolic compound isolated from fresh neem leaves. They also reported other major active compounds such as nimbin, nimbidin, salanin, 6-deacetylnimbin, azadiradione, epoxy-azadiradione *etc.* So, neem leaves are a good choice to reduce silver ions to its zero state[24].

E. scaber is a flowering plant of family compositae and found in moist forests of tropical and subtropical regions *i.e.,* Indian subcontinent, Eastern and Southeast Asia, Northern Australia and tropical Africa. This plant is used as a traditional medicine as astringent agent, cardiac tonic, diuretic, anti microbial *etc. E. scaber* contains a germacranolide sesquiterpene lactone named elephantopin, iso-17,19- dihydro-deoxy elephantopin, 17,19-dihydrodeoxyelephantopin, and 8-hydroxyl Naringenin which mainly acts as anti bacterial agent[25,26].

Our objective was to study the efficiency of the plant extracts *A. indica* and *E. scaber* as a reducing agent for the rapid, single step and eco friendly synthesis of AgNPs as well as the effectivity of the synthesized nanoparticles as an environment friendly mosquitocidal agent. These two plant extract were proved to be excellent reducing agent for the synthesis process. These two plant mediated nanoparticles showed notable larvicidal and pupicidal activity. Moreover, they are supposed to be toxic for pupal hatching when used in sub lethal doses.

2. Materials and methods

2.1. Preparation of nanoparticle synthesis

$AgNO_3$ crystal extra pure was purchased from Merck. Double distilled water was used for synthesis process.

2.2. Collection of plant material

Fresh neem (*A. indica*) leaves (Figure 1a) were collected from the garden of The Deptt. of Environmental science, The University of Burdwan (23°16' N, 87°54' E) and *E. scaber* leaves (Figure 1b) were collected from the adjoining areas of Sonamukhi forest, Bankura (23°30' N, 87°42' E) where it is abundantly available.

Figure 1. a: Neem (*A. indica*); b: Hasti pada (*E. scaber*).

2.3. Preparation of aqueous extract of plants

The collected leaves were surface cleaned with running tap water to remove the debris and other contaminated organic contents, followed by double distilled water. Washed leaves were then air dried for 48 h at room temperature. Aqueous extract of the leaves were prepared by mixing 5 g of dried leaf with 100 mL double distilled water and boiling at 95 ℃ for 10 min with continuous stirring. The filtrates were then filtered by Whatman filter paper. The extracts can be kept in refrigerator for 1 week for further use.

2.4. Synthesis of AgNPs

$AgNO_3$ solution (1 mmol/L) was prepared by dissolving calculated amount of $AgNO_3$ in double distilled water. The plant extracts prepared were used for reduction of Ag^+ to Ag^0. Plant