

Characterization and use of green nanoparticles from the leaves of *Tiliacora acuminata* against larvae of vector *Culex quinquefasciatus* Say

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ABSTRACT: In the present study, it was established that the efficacy of synthesized silver nanoparticles (AgNPs) from leaves of *Tiliacora acuminata* against 3rd instar larvae of the filarial vector, *Culex quinquefasciatus* mosquito. AgNPs was synthesized by 10 g of air-dried leaves of *T. acuminata* crushed and put into a beaker containing 100ml distilled water, boiled for 10 minutes at 60°C temperature and further treated with 10⁻³ M solution of silver nitrate and boiled at 60°C temperature. Nano particles were isolated by centrifuged the solution at 10,000 rpm for 15 minutes. Different concentrations of (1-5 ppm) of synthesized AgNPs were tested against *Cx. quinquefasciatus* larvae. Characterizations of AgNPs were carried out by UV-vis spectrophotometer, X-ray diffraction (XRD), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) procedures. Cent percent mortality was observed at 5 ppm concentration of AgNPs against the 3rd instar larvae of *Cx. quinquefasciatus*. UV-vis spectrophotometer confirmed AgNPs from leaves of *T. acuminata* with a maximum absorption band at 450nm. Scanning electron microscopy confirmed the rough surface of the synthesized nanoparticles. From TEM analysis it was revealed that the shape of nanoparticles was ranging from oval to quasi spherical with an average diameter of 50-70nm. Their crystalline structure was confirmed by X-ray diffraction pattern. It is concluded that AgNPs from leaves of *T. acuminata* established excellent mosquito larvicidal activity against *Cx. quinquefasciatus*.

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KEY WORDS: Silver nanoparticles, shape, size, crystalline structure, SEM, TEM, X-ray diffraction, LC₅₀ value, larvicidal activity

INTRODUCTION

Culex quinquefasciatus Say is vector of *Wuchereria bancrofti* causative agent of filariasis in tropical and subtropical region in world (Hill and Connelly, 2019). They can also transmit pathogens of avian malaria, Western equine encephalitis virus, St Louis encephalitis virus etc. Nearly about 120 million people around the world are under threat of

lymphatic filariasis, 44 million people have chronic symptoms of that disease (Bernhard *et al.*, 2003; Balasubramanian and Nikhil 2013). Every year, nearly about 700 million people are infected by mosquito borne disease, which are a major cause of human death (Tauber, 1997). Use of chemical insecticides on a routine basis also has a negative impact on human and animal health. It also increases insecticidal resistance property among

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vector population, environmental pollution and biomagnifications through food chain (Senthil-Nathan., 2020). Chemical insecticides are becoming increasingly ineffective against these insects; their indiscriminate use or high concentrations can cause severe adverse reactions. An urgent need for a new insecticide capable of overcoming insect resistance patterns and effectively combating disease-causing vectors is required (Balasubramanian and Nikhil 2013). Plants derived insecticides shows to have better result than chemical insecticide. Different phyto-chemical of plant origin including steroids, alkaloids, terpenoids and phenol have been promising result (Mathew *et al.*, 2009; Pavela, 2015). *Terminalia acuminata* belonging to the family Menispermaceae, is a wood climber mainly found in India, Sri lanka and South East Asia. In ayurveda this plant is used as an antidote for snake bites. Leaves of *T. acuminata* are ovate type with tapering at the tips. According to Singha *et al.* (2011), synergistic effect on mosquito larvicidal efficacy as observed when fruits of *Croton caudatus* with flowers of *T. acuminata* were applied on *Cx. quinquefasciatus* larvae. Tiliamasin, a bis benzyloquinoline alkaloids isolated from *T. acuminata* have larvicidal activity on *Cx. quinquefasciatus* mosquito (Sandhanam *et al.*, 2019).

This study aims to find plant-based silver nanoparticles as mosquito larvicidal to control *Cx. quinquefasciatus* as an alternate agent over chemical insecticides. The principal objective of the present study was to evaluate the mosquito larvicidal potential of synthesized silver nanoparticles of leaves of *T. acuminata* against *Cx. Quinquefasciatus* and proper characterization of nanoparticles through FTIR, XRD, SEM and TEM techniques. The present study was carried out at the Department of Zoology, Vivekananda Mahavidyalaya, Burdwan, West Bengal, India. *T. acuminata* plant were collected from bank of Hooghly, Hooghlyghat, West Bengal.

MATERIALS AND METHODS

Mosquito larvae were collected from drains surrounding Vivekananda Mahavidyalaya, Burdwan. Larvae were transferred into a plastic

tray and they were fed with a mixture of dog biscuits and yeast. Mosquito colonies were maintained at a temperature of 25-27°C with 75-85percent relative humidity. For the preparation of an aqueous solution 10 g leaves of *T. acuminata* were crushed and put in 100ml of double distilled water. 10^{-3} M silver nitrate was mixed with an aqueous solution of *T. acuminata* leaves. The mixture was stirred with vortex stirred for 5 minutes. Then the mixture was heated at 60°C temperature for 10 minutes. The colour of solution was changed from light green to the dark brown. It was centrifuged in a Remi research centrifugation instrument at 10000 rpm for 15 min. The pellet was isolated from dark brown solution and it scraped from the centrifuge tube, then dried in a vacuum desiccator. Isolated nanoparticles were used for a bioassay test (Halder *et al.*, 2013).

Larvicidal bioassay was conducted according to the protocol of WHO (2005) with slight modification. Third instar larvae of *Cx. quinquefasciatus* was used for bioassay test (Rawani *et al.*, 2013). Synthesized nanoparticles 1 to 5ppm concentrations were mixed with DMSO and added to each petri-dishes. Twenty five 3rd instar larvae of *Cx. quinquefasciatus* were transferred manually into glass Petri dishes containing 100ml of distilled water. After 24-hour mortalities were recorded against each concentration, the percentage of mortality was corrected using Abbott's formula (Abbott, 1987). All experiment was repeated three times. The control experiment was conducted without silver nanoparticles. Ms excel 2021 was used to determined average larval mortality.

An isolated pellet of silver nanoparticles was examined by using a UV-vis spectrophotometer (Shimadza, 2450, Japan). Synthesized nanoparticles show their unique surface Plasmon resonance (SPR) absorption when it was set to 1mm resolution with a range between 190 - 170 nm (Upstone, 2000). Crystalline property of synthesized nanoparticles could be confirmed by analyzed it through X-ray diffraction procedures. The isolated pellet was converted into powder for the X-ray diffraction test. The shape and size of the synthesized nanoparticles were determined by transmission

electron microscopy. The Techani 20-Philip TEM instrument was used to capture a TEM image and it was operated at 200 KV with beam current of 104.1 μ A. A copper grid containing 300 mesh sizes was used to dry the sample for overnight at 25°C temperature and in a vacuum condition. Scanning electron microscopy (SEM) was used for the determination of surface structure of the synthesized nanoparticles. Before SEM nanoparticles were coated with gold and the SEM instrument was operated at a voltage of 5 KV.

RESULTS AND DISCUSSION

Fresh leaves of *T. acuminata* stimulated bio reduction of Ag^+ to and their concentration was marked by change of solution colour from pale to deep brown colour. Further, it was confirmed by UV-vis spectrometer analyses. Characteristics surface Plasmon resonance band was observed at 450 nm wavelength (Fig. 1) for leaves of *T. acuminata*. Gope *et al.* (2023) observed a similar value. Crystalline structure of nanoparticles was confirmed by XRD pattern (Fig. 2). Different peaks at 2θ values of 37.92, 44.94, 54.4 and 64.21° of leaf *T. acuminata* synthesized AgNPs correspond to crystalline structure of silver nanoparticles. XRD pattern confirmed that leaf silver nanoparticles are crystalline in nature and have an organic coat (Kumar, 2017).

SEM was used to know the surface topography of synthesized nanoparticles (Fig. 3). It indicated that

the surface has a rough texture and is crystalline. Most of the nanoparticles were clumped by attached with each other and a few were free (Malla, 2023). By TEM analysis it was found that synthesized nanoparticles were spherical and quashi spherical in shape (Fig. 4). The average size of the nanoparticles was 50-60 nm in diameter and they were oval and quashi spherical in shape (Halder *et al.*, 2013). Different specifically functional groups in the plant extract of *T. acuminata* which were involved in reduction of Ag^+ to Ag^0 . According to Rodriguez *et al.* (2021) the bulk of the peaks were phyto-chemically present in the plant extract.

The larvicidal activity of synthesized nanoparticles from leaves of *T. acuminata* against 3rd instars larvae of *Cx. quinquefasciatus* revealed 60 percent mortality of 3rd instar larvae at 1ppm concentration of leaf AgNPs whereas 100 percent mortality was recorded at 5 ppm concentration of AgNPs

Mean larvicidal mortality ($\% \pm \text{SE}$ at 24 h) of 3rd instar larvae of *Cx. quinquefasciatus* using synthesized nanoparticles from leaves of *T. acuminata* was 60.00 ± 0.33 at 1ppm, 73.3 ± 0.88 at 2ppm, 86.6 ± 0.33 at 3ppm, 96.6 ± 0.33 at 4ppm and at 5ppm it was 100 ± 0.33 , while the control showed no mortality. LC_{50} value was 0.21ppm in AgNPs of leaves of *T. acuminata*. Probit and regression analyses of mortality rate of *Cx. quinquefasciatus* larvae by nanoparticles established LC_{50} as 0.21ppm under 24 hours of exposure (Regression equation- $Y=047X + 4.93$ and

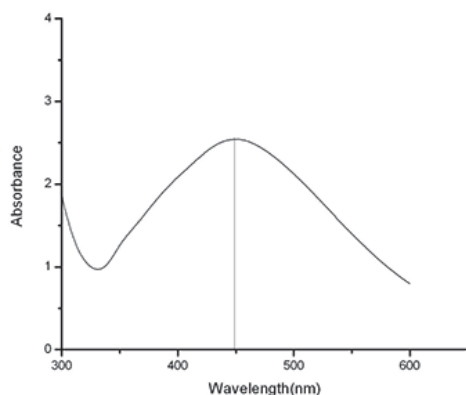


Fig.1 UV-vis spectral analysis of nanoparticles from leaves of *T. acuminata*

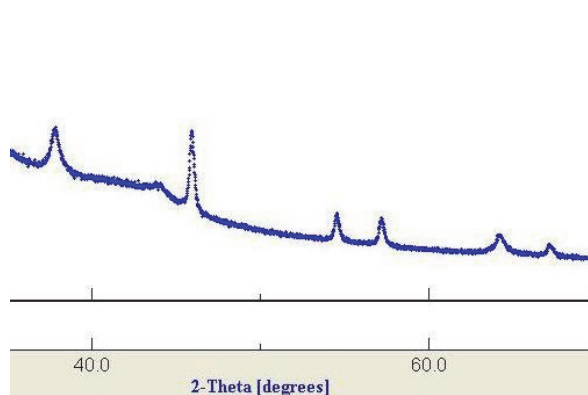


Fig. 2 XRD X-ray diffraction pattern of synthesized nanoparticles from leaves of *T. acuminata*

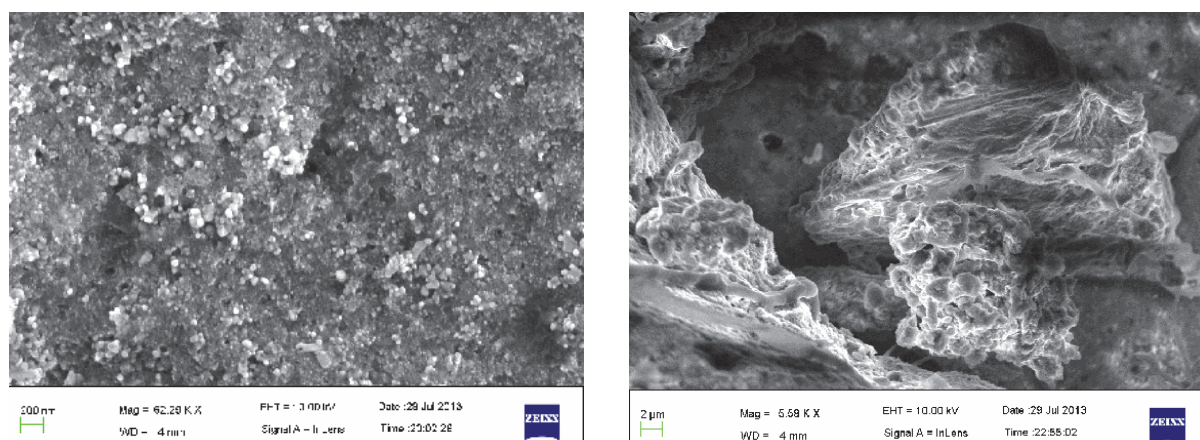


Fig. 3 SEM (scanning electron microscopy) of silver nanoparticles from leaves of *T. acuminata*

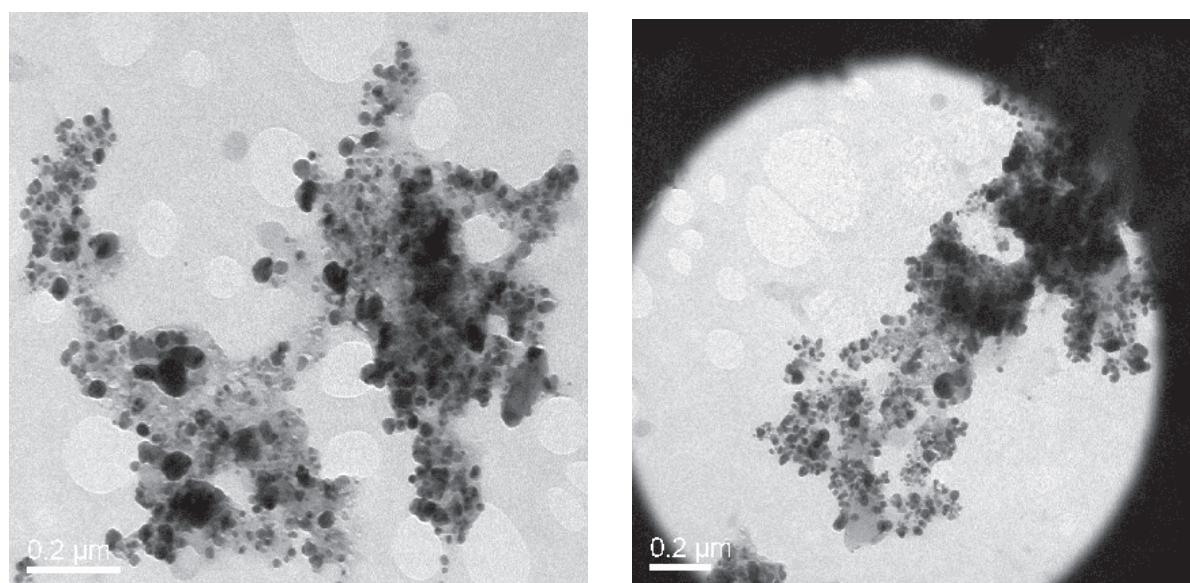


Fig. 4 TEM Transmission electron microscopy of silver nanoparticles from leaves of *T. acuminata*

R-value 0.93). According to Sutthanant *et al.* (2019) LC_{50} value of AgNPs synthesized from leaves of *Curcuma zedoaria* was 36.32ppm against 3rd instars larvae of *Cx. quinquefasciatus*. Gope *et al.* (2023) reported LC_{50} value of AgNPs at 2.09 ppm isolated from the leaf of *Phyllanthus acidus* against the same larvae. *Cx. quinquefasciatus* larvae died after treatment with AgNPs which was due to their deposition in the gut of the mosquito. AgNPs bind to the epithelial membrane of the

midgut and inactivate the midgut enzyme as well as the peroxidase effect, which may cause larval death (Madanagopal *et al.*, 2017). This study identified a safe environmentally friendly product i.e. green synthesized nanoparticles from the leaf of *T. acuminata* as a good larvicidal against *Cx. quinquefasciatus*. It can be an excellent choice as a larvicidal agent for the control of lymphatic filariasis through control of the *Cx. quinquefasciatus* population.

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