Engineering in Agriculture, Environment and Food 9 (2016) 1–8

Contents lists available at ScienceDirect

Engineering in Agriculture, Environment and Food

journal homepage: http://www.sciencedirect.com/eaef

Research paper

Green synthesis of silver nanoparticle using *Achyranthes aspera* and its larvicidal activity against three major mosquito vectors

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ARTICLE INFO

Article history:
Received 12 March 2014
Received in revised form
25 April 2015
Accepted 20 August 2015
Available online 21 August 2015

Keywords:
Achyranthes aspera
Silver nanoparticles
Electron microscopic study
Larvicidal activity
Mosquito vectors

ABSTRACT

Biosynthesis of silver nanoparticles was achieved by novel simple green chemistry procedure using *Achyranthes aspera* leaf extract as a reducing and a capping agent. The present study focuses on larvicidal activity of synthesized silver nanoparticles (AgNPs) against *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi*. Range of concentrations of synthesized AgNPs (2, 4, 6, 8 and 10 mg/ml) and the aqueous extract (100, 50, 25, 12.5 and 6.25 mg/ml) were tested against the fourth instar larvae for 24 h exposure. The maximum efficacy was observed in the synthesized AgNPs against *Cx. quinquefasciatus* (LC_{50} 2.48; LC_{90} 8.14 mg/ml) and *Ae. aegypti* (LC_{50} 3.68; LC_{90} 8.92 mg/ml) whereas aqueous extract found to be effective against *Cx. quinquefasciatus* at a higher concentration. The synthesized AgNPs from *A. aspera* were highly potent than aqueous extract against all the three tested vectors. The synthesized AgNPs were characterized by UV-visible spectrophotometer, SEM, TEM, FTIR and XRD. This revealed a peak at 452 nm in leaf extract of *A. aspera*, indicating the production of AgNPs. The synthesized AgNPs were clearly distinguishable with the respective nanometers and the XRD spectrum confirmed the presence of silver ion and it was compared with the standard spectrum peaks. The FTIR spectra of AgNPs exhibited prominent peaks of organic molecules.

1. Introduction

Nanobiotechnology, an emerging field of nanoscience, utilizes nanobased-systems for various biomedical applications. Nanoparticles play an important role in pharmaceutical, industrial and biotechnological applications. Noble metal nanoparticles such as gold, silver, titanium and platinum are widely applied in nanomedicinal application (Kaviya et al., 2011). In particular, silver nanoparticles are proved to have potential antibacterial, antifungal, anti plasmodial and larvicidal properties (Saxena et al., 2010; Elumalai et al., 2010).

Currently most of the applications of silver nanoparticles in the biotechnology field are as anticancer, antibacterial, larvicidal activity and antifungal agents, in textile engineering, waste water treatment and silver-based consumer products (Suman et al., 2013).

Biological synthesis of nanoparticles has received attention due to a growing need to develop environmentally friendly technologies in material synthesis (Raji et al., 2004). The plant mediated silver nanoparticle is advantageous over chemical and physical methods because it is cost effective and environment friendly method, where it is not necessary to use high pressure, energy, temperature and toxic chemicals (Goodsell, 2004). The use of plants for the fabrication of nanoparticles is a rapid, low cost, ecofriendly and single step method for biosynthesis process (Huang et al., 2007).

Plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. Biosynthetic processes would be more useful if the AgNPs were produced using plants or their extracts in a controlled manner according to their dispersity, shape and size (Kumar and Yadav, 2009).

Mosquitoes are the principal vector of many diseases affecting human beings and other animals. Several mosquito species belonging to genera *Anopheles, Culex* and *Aedes* are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue fever, dengue hemorrhagic fever, chikunkunya and yellow fever.

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http://dx.doi.org/10.1016/j.eaef.2015.08.002
1881-8366/

An. stephensi is recognized as a major vector for malaria in India. This species prefers to breed in small water collections and is responsible for frequent outbreaks of malaria, particularly at construction sites in urban areas (Mittal et al., 2005). *Ae. aegypti* is a cosmotropical species responsible for dengue fever and chikungunya in India, where the number of dengue fever cases has increased significantly in recent years (Harrington et al., 2005; Murugan et al., 2011). *Cx. quinquefasciatus*, the potential vector of lymphatic filariasis, is the most widely distributed tropical disease with around 120 million people infected world wide and 44 million people having chronic manifestation (Bernhard et al., 2003).

Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and has led to resurgences in mosquito populations. It has also resulted in the development of resistance, undesirable effects on non-target organisms and has fostered environmental and human health concern, which has initiated a search for alternative control measures. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents (Shanklin and Cahoon, 1998).

One of the approaches for control of these mosquito borne diseases is the interruption of disease transmission by either killing, preventing mosquitoes bite (by using repellents) or by causing larval mortality in a large scale at the breeding centers of the vectors. This study is concerned with the use of such effective plant source against the larvae of mosquito. These problems have highlighted the need for new strategies for mosquito larval control.

Plants, being a natural source of various compounds, are known to contain larvicidal agents, which may act in combination or independently, hence the need to carry out the studies on the interactions of compounds (Gershenzon and Dudareva, 2007; Wink, 2004). Some phytochemicals act as general toxicants both against adult as well as larval stages of mosquitoes, while others interfere with growth and development (growth inhibitors) or with reproduction as a chemosterilent or produce olfactory stimuli acting as repellent or attractant (ICMR Bulletin, 2003).

The phytochemicals derived from plant sources possess complex chemicals with unique biological activity and can act as larvicides, insect growth regulators, repellents and ovipositional attractants, having deterrent activities as observed by different researchers (Mathivanan et al., 2010).

Due to the growing need for environmental friendly nanoparticles, researchers are using green methods for the synthesis of various metal nanoparticles. Plant extracts are being used as reducing and capping agent for the synthesis of nanoparticles which could be advantageous over phytochemical reduction, heat evaporation, electrochemical reduction, and chemical reduction methods. Several biological systems including bacteria, fungi, and yeast have been used in synthesis of nanoparticles. Silver nanoparticles have attracted intensive research interest because of their important applications as antimicrobial, catalytic, textile fabrics and plastics to eliminate microorganisms. Because of such a wide range of applications, numerous methods concerning the fabrication of silver nanoparticles, as well as various silver-based compounds containing ionic silver or metallic silver have been developed. Synthesized silver nanoparticles from plants such as *Acalypha indica* (Krishnaraj et al., 2010), *Rosa rugosa* (Dubey et al., 2010), *Chenopodium album* (Dwivedi and Gopal, 2010), (Geethalakshmi and Sarada, 2010), *Trianthema decandra* Cycas (Jha and Prasad, 2010), *Stevia rebaudiana* (Yilmaz et al., 2011), *Mangosteen* (Veerasamy et al., 2011), *Macrotyloma uniforms* (Vidhu et al., 2011), *Ficus benghalensis* (Saxena et al., 2012) are proved to possess potential antibacterial, antifungal, anti plasmodial and larvicidal properties (Saxena et al., 2010).

Achyranthes aspera L. (Amaranthaceae) a widely naturalized herbaceous plant is native of Africa and Asia. It is used for the reclamation of wastelands and the seeds and leaves are used for human consumption and in religious ceremonies in India (Ragupathy and Newmaster, 2009). This plant is commonly used in Ayurveda for the treatment of stomach ache, piles, boils and skin eruptions (Fig. 1). The leaf extract is prothyroidic and antiperoxidative in rats (Tahiliani and Kar, 2000). Saponins from the ethyl acetate extract of *A. aspera* leaves have been tested as larvicide against *Ae. aegypti* and *Cx. quinquefasciatus* (Bagavan et al., 2008), and extract of the leaves, flowers and seeds of *A. aspera* have potent biological activity (Khare, 2007). The present study focuses on larvicidal activity of synthesized silver nanoparticles (AgNPs) from aqueous leaf extracts of *A. aspera* against *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi*.

2. Materials and methods

2.1. Plant collection

A. aspera is a weed found in dense clumps along roadsides of Chennai, Tamil Nadu, India. *A. aspera* was identified by (Voucher No; 1212237) by A. Saraswathy, Director, Captain Srinivasan Murthi Institute of Ayurveda and Siddha drug development, Arumbakkam, Chennai-106, India.

2.2. Preparation of plant extract and synthesis of AgNPs

A. aspera leaves were washed thoroughly in tap water for 10 min in order to remove the dust particles and rinsed with deionized water. The aqueous solution was prepared by taking 10 g of washed and finely cut leaves in a 250 mL Erlenmeyer flask along with 100 mL of deionized water and the mixture was boiled at 60 °C for 15 min. This extract was filtered through nylon mesh followed by Millipore hydrophilic filter (0.22 m) and was used for further experiments (Parashar et al., 2009). 5 mL of this aqueous leaf extract was added to 95 mL of 1 mM aqueous $AgNO_3$ solution for the synthesis of AgNPs. A control setup (Silver nitrate) was also maintained without *A. aspera* extract.

2.3. Phytochemical profiling

Phytochemical screening of the sample was carried out as described by (Nweze et al., 2004) and (Senthil Kumar and Reetha, 2009).

Fig. 1. *Achyranthes aspera* (L).