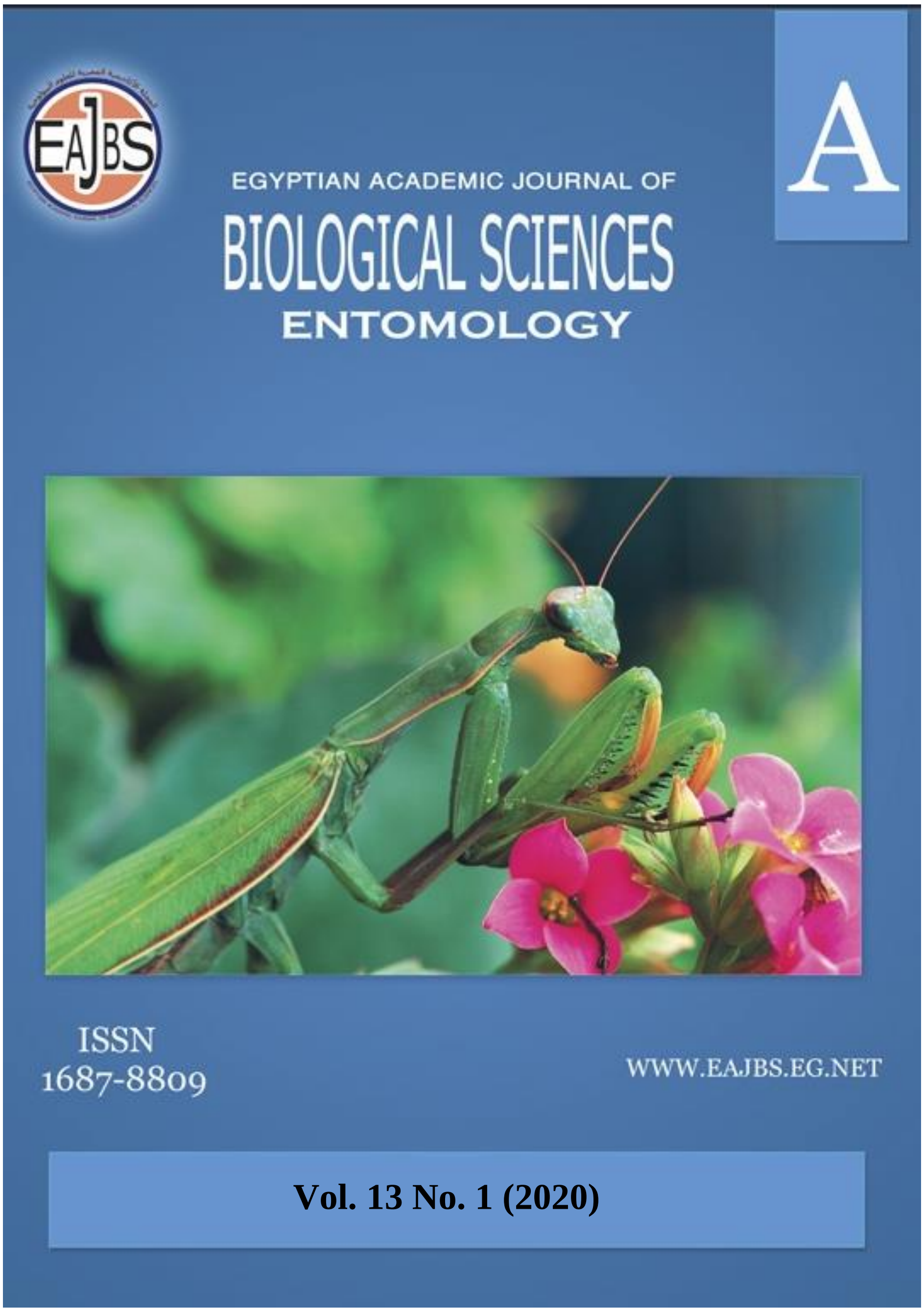

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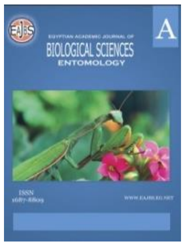

Silver Nanoparticles of *Eucalyptis grandis* as A Possible Insecticide Against Mosquito Vectors.

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ABSTRACT

Mosquito-borne diseases are amongst the most serious threat to humans, especially in tropical countries. Mosquito control has never been easy and has always posed several difficulties like hazards of chemicals used, resistance raised in mosquito populations or cost-effectiveness. Current control methods mainly depend on the use of conventional chemicals, Physical methods, biological methods, and transgenic mosquitoes. Currently, none of these single strategies is fully successful. Unique eco-friendly approaches to achieve mosquito vector control are immediately required. The plant-mediated creation of nanoparticles is helpful over chemical and physical methods since it is cheap, single-step, and easy to use. Recently the number of plant-based compounds have been proposed for efficient synthesis of metal nanoparticles effective against mosquitoes. The present study was an attempt to study the mosquitocidal potential of the bionanomaterial of the bark extract of *Eucalyptis grandis* with silver. The larvicidal activity after 24 hours was found to be LC_{50} = 42 and 35 ppm for the two species of the mosquitoes studied herein.

INTRODUCTION

The tropical world has always been under the burden of vector-borne diseases especially those transmitted through mosquitoes. Epidemic diseases like malaria, filariasis, dengue that are transmitted by different species of mosquitoes can be kept under control only by keeping the population of mosquitoes under control. Several strategies have been continuously evolved with the only aim to kill mosquitoes. The conventional chemicals largely used have caused serious impacts on the ecosystem. Moreover, the emergence of insecticide resistance and harmful effect on non-target organisms has evoked an urgent search for the development of new and improved mosquito control methods and materials too, that are safe and cheap. The products of plant origin have certainly come up with promising results. Such biomaterials with technological improvements can form a base for a sustainable vector management strategy. The botanicals synthesized with nanomaterials have become a new way Insecticides of synthesized nato combat the vector problem.

The word "nano" is derived from a Greek word meaning "dwarf". In technical terms, the word "nano" means 10^{-9}, or one billionth of a meter. Materials with such a tiny size may possess unique properties both physical and chemical to be effectively used in mosquito control methods. "Nano-technology" deals with the application of such particles in biological, physical, chemical, environmental, agricultural, industrial or pharmaceutical science.

A variety of methods are presently used to synthesize different types of silver, gold, silicon, zinc and platinum nanoparticles. Although physical and chemical methods are more popular and widely used for the synthesis of nanoparticles, the related environmental toxicity and non-biodegradable nature of the products limited their applications. So, the "green" way for nanoparticle synthesis from the herbal origin is of great interest due to eco-friendliness, economic prospects and a wide range of applications (Salam et al 2012).

Nanotechnology has a varied application in vector control in the form of nano capsules for herbicide delivery and vector and pest management and nano sensors for pest detection, (Scrinis and Lyons, 2007)

The silver or gold nanoparticles that are synthesized in the laboratories are used along with the plant products to make new insecticides. Thus nanotechnology in this form has contributed in the area of public health to fight against mosquitoes and diseases transmitted through them.

In the present study, attempt has been made to assess the effectiveness of the bionanomaterial of silver and aqueous extract of bark of *Eucalyptis grandis.*

Eucalyptus grandis attains a height of 45-55 m, usually with an excellent trunk and a wide-spreading, rather thin crown; most of the bark and branches are smooth, white or silvery, sometimes greenish, rough on the stem, smooth above, debark easily. Juvenile leaves are petiolate, opposite for several pairs then alternate, ovate up to 16 x 8.5 cm, green to dark green and slightly wavy; adult leaves are petiolate, alternate stalked, lanceolate to broad lanceolate, up to 15 x 3 cm, green on topside and pale green on underside, slightly wavy, with a long point. Inflorescence axillary and simple, 7 flowered; peduncules flattened, to 1.8 cm long; buds have a bluish bloom. Fruit or seed capsules several, short-stalked, pear-shaped or conical, slightly narrowed at the rim, thin, 8 x 6 mm, with a whitish waxy coating, narrow sunken disc, and 4-6 pointed, thin teeth, slightly projecting and curved inward, persisting on twigs. The main constituents of the oil of the *E. grandis* are α-Pinene (29.69%), p-Cymene (19.89%), 1,8-cineole (12.80%), α-Terpineol (6.48%), Borneol (3.48%) and 3.14% D-Limonene (Oluwagbemiga et al 2013).

The insecticidal properties of the selected plant material used in the form of silver nanomaterial were assessed for the mosquito larvicidal activity in laboratory conditions.

MATERIALS AND METHODS

Plant Material:

The bark of *Eucalyptus grandis* was collected from the Akurdi region of Pune District of Maharashtra in September 2017.

Extract Preparation:

The bark was washed with distilled water for removing the dirt and mud particles if any. The bark was air-dried and converted into powder. Ten gm of bark powder was then placed in a 250 mL of deionized water to make a suspension. This mixture was boiled at 65^{o} C, for 5 min, decanted and filtered through Whatman filter paper no 1.

Bionanoparticles Synthesis:

After obtaining the aqueous extract of bark, the filtrates were treated with aqueous 1 mM AgNO3 solutions in an Erlenmeyer flask and incubated at room temperature. The formation of AgNPs was indicated by the brown coloration of the solutions. Synthesis of the AgNPs was confirmed by sampling the reaction mixture at regular intervals, and the absorption maxima were scanned by UV-Vis spectra, at the wavelength of 350–750 nm.

Mosquito Rearing:

The mosquitoes, *A. Aegypti* and *C.quinquefasciatus* were reared (as per WHO guidelines) in the Department of Zoology, Prof. Ramkrishna More College, Akurdi Pune -44. Mosquitoes were kept at (28± 2) °C, 75%-85% relative humidity (RH), with a photoperiod of 12 h light, 12 h dark. The adults were reared in separate metal cages 24”x24”x12” with a cotton sleeve at one end to have easy access to the culture. The larvae were fed on dog biscuits and yeast powder. Adults were provided with 10% sucrose solution and blood meal.

Larvicidal Bioassay:

The larvicidal activity was performed according to the guidelines for laboratory and field testing of mosquito larvicides published by WHO (who/cds/whopes/gcdpp/2005.13), with minor modifications.

Initially, the mosquito larvae were exposed to a wide range of test concentrations and a control to find out the activity range of the extracts under test. Batches of 25 third-instars larvae of the mosquitoes were placed in a small plastic container with 200 ml dechlorinated water Small, unhealthy or damaged larvae were removed and replaced. The depth of the water in the containers was between 8 cm and 10 cm. Larval bioassays were carried out using the desired concentration of the extract. Five replicates per concentration and 6 concentrations in the activity range of the extract were used. Larval mortality was recorded after 24 h exposure and was calculated using the following formula:

Percentage mortality = Number of dead larvae / Number of larvae introduced x 100

Results were subjected to statistical analysis.

RESULTS AND DISCUSSION

The disadvantages of the conventional chemicals used to control mosquitoes have made it imperative to search for new safer and cheaper methods. Plant products due to their metabolites have always been preferred to be considered as an eco-friendly alternative to the conventional chemical method of insect control. The effectiveness of the plant products can certainly be improved by synthesizing bionanomaterial of such plant material with silver or other suitable compounds/metal.

In the present investigation, AgNP has been synthesized by using the bark of *Eucalyptus grandis*. The efficacy of synthesized NPs has been tested against the larvae of the dengue vector and filariasis vector. The result reveals a significant larvicidal activity of the synthesized bionanomaterials against both the mosquito species(Table 1). The results obtained are certainly promising and the effectiveness at a low concentration will certainly prove to be eco-friendly.

Table 1: Larivicidal activity of AgNP against *A.aegypti* and *C.quinquefasciatus I* after 24 hours.

Mosquito	LC50±SE (ppm)	95% Confidential limit		Regression equation	LC_{90} (ppm)
		LCL	UCL		
A.aegypti	42±0.77	31	71	Y=2.32X-2.06	69
C.quinquefasciatus	35±1.05	23	57	Y=1.32X-3.21	61

An economically viable and “green chemistry” approach for biological synthesis of silver nanoparticles using aqueous leaf extract of *P. dulce* has been reported as larvicidal activity against the *C. quinquefasciatus* previously (Raman et. al 2012). The larvicidal activity of biogenic nanoparticles against filariasis causing *Culex* mosquito vector has also been evaluated before (Dhanasekaran and R. Thangaraj 2013). Rajakumar *et al*. (2011) reported the use of silver nano particles synthesized from leaf extract of *Eclipta prostrata*, to control 4th instar larvae of *Culex quinquefasciatus* (*Cx.quinquefasciatus*) and *Anopheles subpictus* (*An. subpictus*). The highest mortality was calculated at 24 h in synthesized silver nanoparticles against *Cx. quinquefasciatus*(LC_{50}=4.56 mg/L; LC_{90}=13.14 mg/L) than *An. subpictus* (LC_{50}=5.14 mg/L; LC_{90}=25.68 mg/L). Sareen *et.al*.(2012/ had reported the larvicidal efficacy of silver nanoparticles synthesized from aqueous leaf extract of *Hibiscus rosasinensis* against the larvae of *Aedes albopictus* (Saren et al 2012). After 12 hours of exposure, 50% mortality was observed and after 16 hours of treatment, 90% mortality was calculated in 1.0 mg/L. 100% mortality was recorded in 5.0 mg/L of silver nanoparticles after 3 hours of exposure. Jayaseelan *et al*.(2011) observed the larvicidal potentiality of synthesized silver nanoparticles using leaf aqueous extract of *Tinospora cordifolia* Miers against *An. subpictus* and *Cx. Quinquefasciatus* (Jayaseelan et. al 2011). The maximum efficacy was found against the larvae of *An. subpictus* (LC_{50}=6.43 mg/L).

These results reveal that the green, biological synthesis of silver particles has the prospective to be employed as a good, rapid, eco-friendly method for the management of the mosquito population. After few more experiments and field trials, there can be a possibility to use this nanomaterial in mosquito control.

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