# RESEARCH PUBLICATIONS

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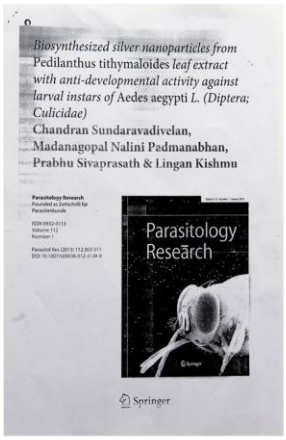
# RESEARCH PUBLICATIONS IN SIJ JOURNALS



*Biosynthesized silver nanoparticles from* Pedilanthus tithymaloides *leaf extract with anti-developmental activity against larval instars of* Aedes aegypti *L. (Diptera; Culicidae)*

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Parasitology Research
Founded as Zeitschrift für Parasitenkunde

ISSN 0932-0113
Volume 112
Number 1

Parasitol Res (2013) 112:303-311
DOI 10.1007/s00436-012-3138-9

Parasitology Research

Springer

Author's personal copy

Parasitol Res (2013) 112:303–311
DOI 10.1007/s00436-012-3138-9

ORIGINAL PAPER

**Biosynthesized silver nanoparticles from *Pedilanthus tithymaloides* leaf extract with anti-developmental activity against larval instars of *Aedes aegypti* L. (Diptera; Culicidae)**

**Chandran Sundaravadivelan · Madanagopal Nalini Padmanabhan · Prabhu Sivaprasath · Lingan Kishmu**

Received: 4 August 2012 / Accepted: 19 September 2012 / Published online: 30 September 2012


**Abstract** Mosquitoes transmit dreadful diseases to human beings wherein biological control of these vectors using plant-derived molecules would be an alternative to reduce mosquito population. Aqueous leaf extract and green synthesized silver nanoparticles (Ag NPs) from *Pedilanthus tithymaloides* (L.) Poit. were investigated for their efficacy against the dengue vector *Aedes aegypti* L. (Diptera; Culicidae). The biologically synthesized Ag NPs were characterized by UV-vis spectrum, X-ray diffraction, Fourier transform infrared, and surface characteristics by atomic force microscopy. Further, on exposure of the larvae to varying concentrations of aqueous leaf and Ag NPs for 24 h, these Ag NPs showed 100 % mortality from first to fourth instars and pupae of *A. aegypti* at 0.25 %, which is the highest concentration, tested, wherein it was the lowest concentration of aqueous leaf extract alone which showed only 10–18 % of mortality. Lethal concentration ($LC_{50}$) values of Ag NPs against the larval and pupal stages were 0.029, 0.027, 0.047, 0.086, and 0.018 % with no mortality in control. These results suggest that the use of *P. tithymaloides* silver nanoparticles can be a rapid, environmentally safer bio-pesticide which can form a novel approach to develop effective biocides for controlling the target vector.

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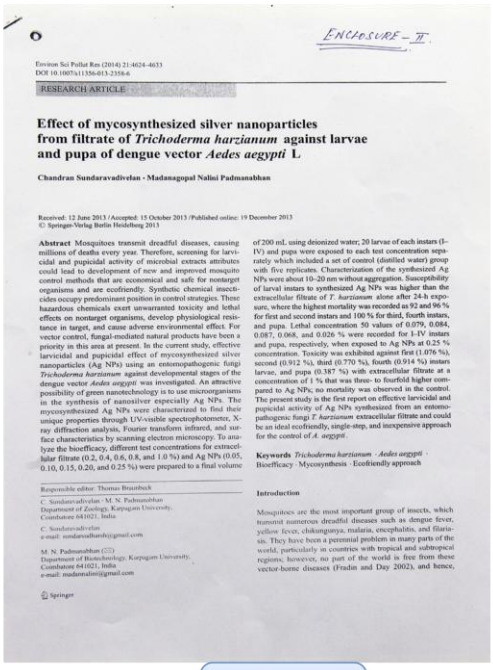
**Introduction**

Mosquitoes occupy different environmental niches and are distributed in diverse habitats. In most regions, there are many species of mosquito, but only a few are of major concern as significant vectors of diseases to humans and cattle. Worldwide, in recent years, mosquitoes are medically the most important group of insects among arthropods responsible for vector-borne diseases like yellow fever, chikungunya fever, dengue fever, dengue hemorrhagic fever, dengue shock syndrome, malaria, Japanese encephalitis, and lymphatic filariasis. *Aedes aegypti*, a vector of dengue that carries the arbovirus responsible for these viral diseases, is widely distributed in the tropical and subtropical zones. Chikungunya, a viral disease caused by both *A. aegypti* and *Aedes albopictus*, was recently concerned as an important public health problem in India and other countries like Senegal, West Africa (Yamar et al. 2005). As per WHO (1984), globally about 90 million people were infected with *Wuchereria bancrofti*, the vector parasite which is the world's most important vector-borne dreadful disease. The problem is more severe in the rural areas especially those associated with well-irrigated agroecosystem, several agricultural practices, poor sanitation facilities, and lack of medical facilities enhancing the diversity and density of the population of mosquitoes and occurrence of vector-borne diseases. For successful implementation of vector management programs, adequate knowledge about the mosquito species and their vector role is essential to maintain public health (Sundaravadivelan et al. 2011). The availability of water sources and favorable climatic conditions create the suitable breeding sites for mosquitoes and their relative abundance among the mosquito populations, which are vectors of West Nile Virus, and maintain their enzootic cycles (Medlock et al. 2006).



**IF : 2.812**



ENCLOSURE – II

Environ Sci Pollut Res (2014) 21:4624–4633
DOI 10.1007/s11356-013-2358-6

RESEARCH ARTICLE

**Effect of mycosynthesized silver nanoparticles from filtrate of *Trichoderma harzianum* against larvae and pupa of dengue vector *Aedes aegypti* L**

**Chandran Sundaravadivelan · Madanagopal Nalini Padmanabhan**

Received: 12 June 2013 / Accepted: 15 October 2013 / Published online: 19 December 2013


**Abstract** Mosquitoes transmit dreadful diseases, causing millions of deaths every year. Therefore, screening for larvicidal and pupicidal activity of microbial extracts attributes could lead to development of new and improved mosquito control methods that are economical and safe for nontarget organisms and are ecofriendly. Synthetic chemical insecticides occupy predominant position in control strategies. These hazardous chemicals exert unwarranted toxicity and lethal effects on nontarget organisms, develop physiological resistance in target, and cause adverse environmental effect. For vector control, fungal-mediated natural products have been a priority in this area at present. In the current study, effective larvicidal and pupicidal effect of mycosynthesized silver nanoparticles (Ag NPs) using an entomopathogenic fungi *Trichoderma harzianum* against developmental stages of the dengue vector *Aedes aegypti* was investigated. An attractive possibility of green nanotechnology is to use microorganisms in the synthesis of nanosilver especially Ag NPs. The mycosynthesized Ag NPs were characterized to find their unique properties through UV-visible spectrophotometer, X-ray diffraction analysis, Fourier transform infrared, and surface characteristics by scanning electron microscopy. To analyze the bioefficacy, different test concentrations for extracellular filtrate (0.2, 0.4, 0.6, 0.8, and 1.0 %) and Ag NPs (0.05, 0.10, 0.15, 0.20, and 0.25 %) were prepared to a final volume of 200 mL using deionized water; 20 larvae of each instars (I–IV) and pupa were exposed to each test concentration separately which included a set of control (distilled water) group with five replicates. Characterization of the synthesized Ag NPs were about 10–20 nm without aggregation. Susceptibility of larval instars to synthesized Ag NPs was higher than the extracellular filtrate of *T. harzianum* alone after 24-h exposure, where the highest mortality was recorded as 92 and 96 % for first and second instars and 100 % for third, fourth instars, and pupa. Lethal concentration 50 values of 0.079, 0.084, 0.087, 0.068, and 0.026 % were recorded for I–IV instars and pupa, respectively, when exposed to Ag NPs at 0.25 % concentration. Toxicity was exhibited against first (1.076 %), second (0.912 %), third (0.770 %), fourth (0.914 %) instars larvae, and pupa (0.387 %) with extracellular filtrate at a concentration of 1 % that was three- to fourfold higher compared to Ag NPs; no mortality was observed in the control. The present study is the first report on effective larvicidal and pupicidal activity of Ag NPs synthesized from an entomopathogenic fungi *T. harzianum* extracellular filtrate and could be an ideal ecofriendly, single-step, and inexpensive approach for the control of *A. aegypti*.

**Keywords** *Trichoderma harzianum* · *Aedes aegypti* · Bioefficacy · Mycosynthesis · Ecofriendly approach

Responsible editor: Thomas Brandsak

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**Introduction**

Mosquitoes are the most important group of insects, which transmit numerous dreadful diseases such as dengue fever, yellow fever, chikungunya, malaria, encephalitis, and filariasis. They have been a perennial problem in many parts of the world, particularly in countries with tropical and subtropical regions; however, no part of the world is free from these vector-borne diseases (Fradin and Day 2002), and hence,



**IF : 2.612**

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading

# Biolarvicidal effect of phyto-synthesized silver nanoparticles using *Pedilanthus tithymaloides* (L.) Poit stem extract against the dengue vector *Aedes aegypti* L. (Diptera; Culicidae)

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ARTICLE INFO

*Article history:*
Received 5 September 2012
Received in revised form 10 September 2012
Accepted
Available online

*Keywords:*
*Pedilanthus tithymaloides*
Stem extract
Silver nanoparticles
Anti-developmental activity
*Aedes aegypti*
Bio-pesticides

ABSTRACT

**Objective:** To identify and develop the alternative novel nanosized biopesticide for quick delivery with potential action using *Pedilanthus tithymaloides (P. tithymaloides)* (L.) Poit aqueous stem extract for the control of mosquito vector *Aedes aegypti (A. aegypti)*, responsible for dreadful diseases like Chikungunya and Dengue. **Methods:** Biologically synthesized silver nanoparticles (Ag NPs) were characterized through ultraviolet-visible (UV-Vis) spectrophotometry, fourier transform infrared (FTIR), X-ray diffraction analysis (XRD), energy dispersive X-ray analysis (EDX), atomic force microscopy (AFM). **Results:** Responsible peak at 463 nm was noticed in UV-Vis spectrum and spherical shaped, crystalline nature of particles was recorded under XRD analysis. FTIR showed the proteins, aromatic and aliphatic amines corresponding peaks to be presence of responsible compounds to produce nanoparticles in the reaction mixture. Presence of silver metal and 15-30 nm sized particles were recorded using EDX and AFM. In the treatments, appreciable toxicity effect of silver nanoparticles was observed and their $LC_{50}$ values 0.046, 0.051, 0.046, 0.167 and 0.054 % (I-IV instars and pupa) were observed at 0.25 % concentration level, which was the lowest concentration compare to aqueous stem extract alone and its LC50 values 1.529, 1.282, 1.450, 2.210 and 1.455 % were also noticed after 24 h exposure. **Conclusions:** This novel approach would be effective to develop the nanosized biopesticide using natural resources as eco-friendly approach against target dreadful vector and able to reduce/avoid the development of resistant in the responsible vector.

## 1. Introduction

Mosquitoes are responsible for transmitting various dreadful diseases like, yellow fever, chikungunya fever, dengue fever, dengue hemorrhagic fever, dengue shock syndrome, malaria, *Japanese encephalitis* and lymphatic filariasis. Viral diseases, chikungunya and dengue caused by both *Aedes aegypti* and *A. albopictus*. Nowadays, mosquito control is severe problem and cause thread due to the emergence of resistance in target and non-target organisms by continuous use of conventional synthetic insecticides. So, there is the constant need to develop and utilize the biological agents to control mosquito larvae in their breeding sites. Earlier researchers have been reported the considerable larvicidal and pupicidal effect using plant extracts of *Azadirachta indica*[1], *Mimosa pudica*[2], *Citrullus colocynthis*[3], *Cassia obtusifolia*[4], *Thymus vulgaris*[5], *Rauwolfia serpentine*[6], *Eugenia jambolana*[7], *Acalypha alnifolia*[8]and *Knema attenuate*[9]against the filarial, malarial and dengue vectors, *Culex quinquefasciatus*, *Anopheles stephensi*, *A. aegypti* and *A. albopictus*.

*Pedilanthus tithymaloides* (L.) Poit belongs to family Euphorbiaceae. It is a small annual shrub commonly available in tropical and sub-tropical regions. The tribal people call it "Moroo Nak" (Marma) and "Jew's Slipper" in English and "Kandai Kalli" in Tamil, which grows in wastelands in northern and eastern parts of India[10]. The stem is dark green in color, fleshy and also secretes latex. The plant parts are qualitatively well investigated for presence of triterpenoides, long-chain alcohols, newly isolated compounds like, 5'-S-methylthioadenosine, pyrogallol, 1,4-dihydroquinone, nicotinamide, prolin, butyric, 2-hexenoic, caproic, cinnamic, dihydroxy

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Chemico-Biological Interactions 207 (2014) 67–73

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

# Hematological, biochemical and enzymological responses in an Indian major carp *Labeo rohita* induced by sublethal concentration of waterborne selenite exposure

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ARTICLE INFO

*Article history:*
Received 6 July 2013
Received in revised form 28 September 2013
Accepted 22 October 2013
Available online 30 October 2013

*Keywords:*
Selenium
Sublethal toxicity
Hematological
Biochemical
Enzymological parameters
*Labeo rohita*

ABSTRACT

Selenium (Se) pollution in aquatic ecosystem is an environmental issue throughout the world. Elevated concentrations of inorganic Se from agricultural and industrial processes may cause adverse biological effects in aquatic organisms such as fish. In the present study, *Labeo rohita* an Indian major carp were exposed to sublethal concentration of Se (sodium selenite) for 35 days and certain hematological, biochemical and enzymological parameters were estimated. The median lethal concentration of waterborne sodium selenite ($Na_2SeO_3$) to *L. rohita* was found to be 23.89 mg $L^{-1}$ for 96 h. During sublethal (2.38 mg $L^{-1}$) treatment, hematological and biochemical parameters such as hemoglobin (Hb) (except 14th day), hematocrit (Hct), erythrocyte (RBC) count and protein levels were found to be decreased in Se treated fish whereas leucocyte (WBC) count and glucose level increased in Se treated fish throughout the study period. The enzymatic parameters such as glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and lactate dehydrogenase (LDH) activities were found to be increased in liver of Se treated fish *L. rohita*. A biphasic response was observed in the value of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The alterations of these parameters can be used as suitable biomarkers in monitoring of selenium in the aquatic environment and to protect aquatic life.



## 1. Introduction

Metals and their components are considered to be a major ecological health concerns worldwide [1–3]. Selenium (Se) is a naturally occurring non metallic element and exists in the environment in different forms; they are selenide (Se [II]), elemental selenite (Se [IV] or $SeO_3^{2-}$) and selenate (Se [VI] or $SeO_4^{2-}$) [4–6]. The inorganic and/or organic selenium compounds are essential for the development of the acquired immune system in many organisms [7]. Moreover, Se is an important element of many protein molecules with diverse physiological functions [8,9]. However, anthropogenic activities such as metal mining and smelting, coal combustion, and agriculture may leads to large quantities of Se in aquatic environment [10,11]. High concentrations of Se were detected in surface water, sediments, and aquatic organisms in different parts of the world and are an environmental issue throughout the world [9]. In the dynamic aquatic ecosystem selenium can be cycled back into the biota and continue to high levels for many years [12]. Furthermore, Se can be ingested by organisms or binds with particulate matter, or be free without binding to any substance [13]. The accumulation of selenium in aquatic organisms may leads to hematological alterations, tissue pathology, reproductive failure, teratogenic deformities and cytotoxicity [11,14,15].

In aquatic ecosystem, Se contamination is more challenging than any other chemical contamination [16]. Moreover, all chemical form of Se has different toxicological and biological properties [17,18]. Generally, fish model is widely used to study Se toxicity due to their higher nutritional requirement of selenium than mammals [8]. Moreover, the biochemical pathways involved in selenium metabolism in fish are almost unknown. In toxicological assay, hematological and biochemical parameters are widely used as health indicators, because they react before the toxicant enters into the body of the organism [19,20]. Hematological parameters such as hematocrit (Hct), hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), are used to assess the functional status of the oxygen carrying capacity and also indicate secondary responses of an organism to irritants such as metals [21,22]. The erythrocyte blood indices such as MCV, MCH and MCHC are widely used to diagnose anemia in animals under stress conditions [23].

Likewise, biochemical parameters such as protein and glucose are highly sensitive to stress conditions and often used in detection of status of stress condition [24]. Furthermore, enzymological parameters are frequently used to assess the impact of metals in

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0009-2797/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.cbi.2013.10.018