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Physiological and Molecular Plant Pathology xxx (2017) 1–9

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

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

A novel photo-biological engineering method for *Salvia miltiorrhiza*-mediated fabrication of silver nanoparticles using LED lights sources and its effectiveness against *Aedes aegypti* mosquito larvae and microbial pathogens

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ARTICLE INFO

Article history:
Received 14 February 2017
Received in revised form
21 March 2017
Accepted 29 March 2017
Available online xxx

Keywords:
Salvia miltiorrhiza
LED
Nanoparticles
Characterization
Antibacterial
Zika virus

ABSTRACT

In this study, *Salvia miltiorrhiza*-synthesized Ag nanoparticles (AgNPs) fabricated using sunlight or various LED lights were studied for their biophysical features and evaluated as larvicides against *Aedes aegypti* mosquitoes and growth inhibitors on different species of microbial pathogens. AgNPs production post-exposure to sunlight or different LED light conditions (i.e. blue, red, green, and white) was confirmed by characteristic surface Plasmon resonance (SPR) at maximum λ of 430, 420, 460, 450, and 460 nm, respectively. Optimization of pH, reducing extract concentration, metal ion concentration and time elapsed from the nano-biosynthesis was achieved. High-resolution transmission electron microscopy (HR-TEM) showed that most AgNPs was spherical, triangular and oval, with average size of 18.5, 28.02, 50.22, 16.26 and 10.12 nm for white, green, red, blue and sunlight, respectively. XRD confirmed the all the obtained AgNPs showed face centered cubic (fcc) crystal lattice. FT-IR analysis of all synthesized AgNPs indicated the involvement of phenol, amine, hydroxyl and amino groups in the reduction of nano-Ag. All tested AgNPs inhibited the growth of *Brevibacterium linens* (KACC-14346), *Propionibacterium acnes* (KACC 11946), *Staphylococcus aureus* (KACC-10768) and *Staphylococcus epidermidis.* As a general trend, larvicidal assays conducted on dengue and Zika virus vector *Aedes aegypti* showed that, after 48 h of exposure, the toxicity achieved by sunlight-fabricated AgNPs was slightly higher if compared to AgNPs fabricated using various LED lights. Overall, our research highlighted the importance of abiotic parameters, with special reference to light condition, during green nanosynthesis of antimicrobials and larvicides.

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1. Introduction

Recently, green nanotechnology received emphasis for various industrial applications including biology, medicine, energy, and electronics [1–5]. Eco-friendly metallic nanoparticles can be

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http://dx.doi.org/10.1016/j.pmpp.2017.03.010
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Please cite this article in press as: J.-H. Lee, et al., A novel photo-biological engineering method for *Salvia miltiorrhiza*-mediated fabrication of silver nanoparticles using LED lights sources and its effectiveness against *Aedes aegypti* mosquito larvae and microbial pathogens, Physiological and Molecular Plant Pathology (2017), http://dx.doi.org/10.1016/j.pmpp.2017.03.010

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obtained exploiting plant extracts, bacteria and fungi as reducing and capping agents [6]. Currently, metallic nanoparticle is obtained from several metals, i.e. silver, gold, platinum, palladium, lead, and titanium, through green-based reducing routes [3,7,8].

In tropical and sub-tropical regions, every year mosquito-borne diseases infect millions of peoples, with special reference to malaria and yellow fever [9,10]. In addition, emerging arboviral diseases, such as dengue, West Nile, chikungunya, and Zika virus. Mosquitoes also transmit pathogens and parasites of veterinary interest, both for livestock, pets and wildlife, including dog heartworm and Eastern equine encephalitis [11].

To control these diseases, an efficient and timely management of mosquito populations is important. Currently, chemical and microbial insecticides still represent the pest populations [12]. To control mosquito vectors with eco-friendly mode, several botanical pesticides have been proposed [13]. In addition, botanical products, as well as microbial cultures, can be used as reducing and stabilizing agents for the fabrication of nano-mosquitocides [3,7].

Furthermore, multi-drug resistant bacteria are now widespread and represent a major public health concern, due to frequent use of antibiotic during therapy. Thus, the development of effective bactericides with multiple mechanisms of action in required. To overcome this problem, researchers are now focusing on metal nanoparticles as bactericides. Silver covered a prominent place as antimicrobial agent to treat several pathogen infections. Employing different botanical products often lead to the production of nanoparticles with different toxicity and physical features (see Benelli [8] for a recent review).

Salvia miltiorrhiza Bunge belongs to family Lamiaceae. It is a perennial herb [14]. "Danshen" is the common name of the *S. miltiorrhiza* root, which is frequently used in Chinese traditional medicine [15] to treat treatment hyperlipidemia, cerebrovascular, acute ischemic stroke diseases, cardiovascular, tumor and cancer [14]. Two groups of active constituents of danshen have been already described by Li et al. [14], as lipid-soluble tanshinones, while the other is composed by water-soluble phenolic acids. More than twenty phenolic acids have been isolated from danshen [16].

In this study, *S. miltiorrhiza*-synthesized Ag nanoparticles (AgNPs) fabricated using sunlight or various LED lights (i.e. blue, red, green, and white LED; Velmurugan et al. [6], were studied for their biophysical features and evaluated as larvicides against *Aedes aegypti* mosquitoes and growth inhibitors on different species of microbial pathogens, including *Brevibacterium linens* (KACC-14346), *Propionibacterium acnes* (KACC 11946), *Staphylococcus aureus* (KACC-10768) and *Staphylococcus epidermidis*.

2. Materials and methods

2.1. *S. miltiorrhiza* aqueous extract

Dried roots of *S. miltiorrhiza* were provided by Department of Oriental Medicine, Chonbuk National University, Iksan Campus, South Korea. *S. miltiorrhiza* roots were washed with sterile nanopure water (conductivity = 18 μΩ/m, TOC <3 ppb) (Barnstead, Waltham, MA, USA). Dried roots were powdered by using mechanical grinder, sieved to get uniform size. 15 g of sieved powder was taken, mixed with 100 mL of nanopure water, kept in hot plate 100 °C for 1 h, and then filtered by Whatman No. 1 filter paper. Filtered *S. miltiorrhiza* root extract (SMRE hereafter) was stored at 4 °C until testing.

2.2. LED light sources and nanoparticle synthesis optimization

For the reaction mixture, 10 mL of SMRE was added to 40 mL of 1 mM $AgNO_3$ (Dae Jung Chemicals, Seoul, South Korea) and exposed to sunlight or different LED lights (i.e. blue, red, green, and white) at room temperature for 30 min (Fig. 1). LED light chamber was supplied by ODTech (South Korea). The obtained product was cleaned by routine centrifugation at 12,000 rpm for 15 min using Hanil Supra 22 K (Hanil, Incheon, South Korea). Final product was freeze dried to obtain powder for further investigation.

UV–Vis absorption spectra were measured using a UV-Visible

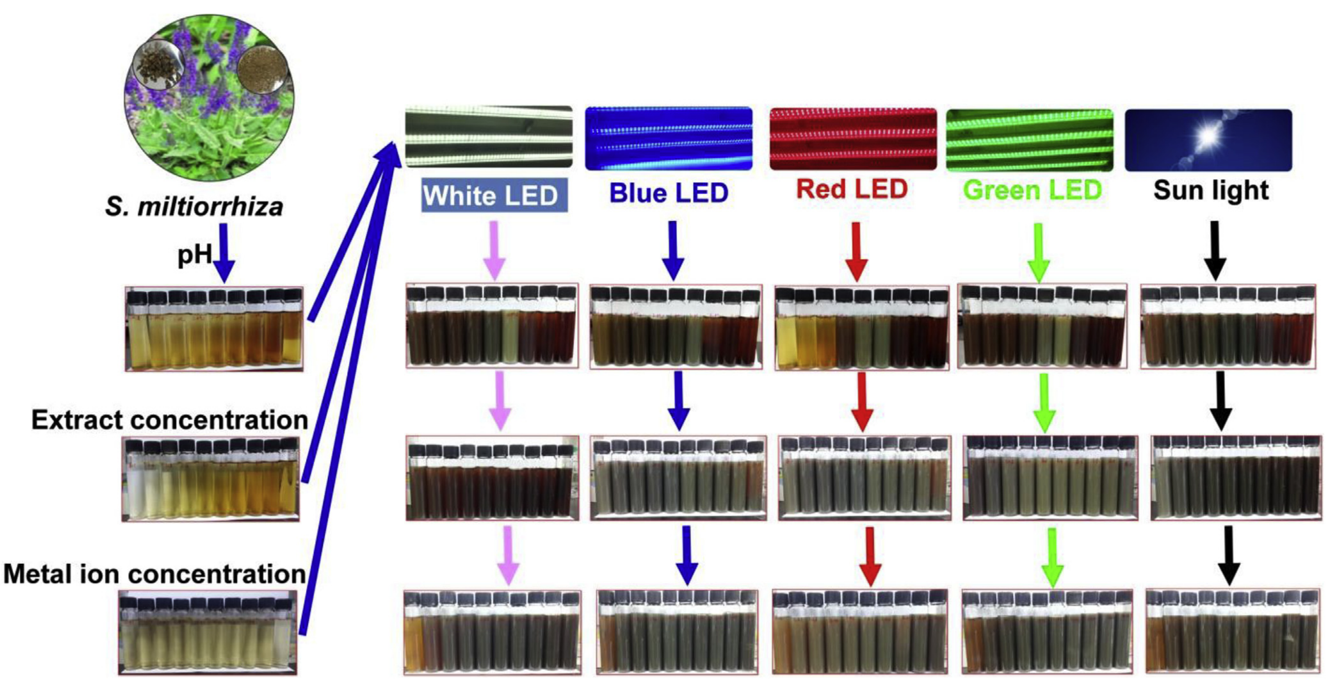

Fig. 1. Impact of pH, reducing extract concentration and Ag^+ concentration on color changes in aqueous suspensions of *Salvia miltiorrhiza*-synthesized Ag nanoparticles fabricated in presence of sunlight or different LED lights.

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