GREEN SYNTHESIS OF SILVER NANOPARTICLES MEDIATED BY NEEM LEAVES EXTRACT AS ANTIFUNGAL AGENT AGAINST FUNGI ISOLATED FROM WATER TREATMENT

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We hereby declare that We have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science.

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Pencemaran kulat dalam sistem pengedaran air minum boleh menyebabkan masalah rasa dan bau, yang mana akhirnya boleh mempengaruhi kualiti air yang dihantar dan boleh menyebabkan ancaman kesihatan kepada orang awam. *Rhodotorula mucilaginosa* dan *Aspergillus* sp. adalah antara kulat patogen berbahaya yang ditemui dalam air dan boleh menjangkiti individu yang mempunyai sistem imun yang lemah. Penggunaan rawatan kimia untuk menghapuskan patogen ini juga boleh meninggalkan residu penyahjangkitan yang toksik dan mungkin terlalu mahal di beberapa negara. Keadaan ini menimbulkan minat untuk membangunkan disinfektan semulajadi yang lebih efektif dengan toksisiti yang lebih rendah dalam rawatan air. Kajian ini menggunakan ekstrak daun semambu (NLE) dan nanopartikel perak yang disintesis secara hijau melalui ekstrak daun semambu (NLE-AgNPs) sebagai disinfektan alternatif semulajadi atau agen antikulat terhadap kedua-dua kulat patogen. Pembentukan NLE-AgNPs disahkan melalui beberapa proses pencirian termasuk analisis spektroskopi sinar ultra violet-visible (UV-vis), spektroskopi inframerah transformasi Fourier (FTIR), difraksi sinar X (XRD), mikroskop elektron pengimbas pelepasan medan (FESEM) dan mikroskop transmisi elektron (TEM). NLE-AgNPs menunjukkan puncak spektra UV-vis sekitar 421 nm, bersifat kristal, sebaran seragam dan mempunyai morfologi sfera dengan saiz purata diameter 20.13 - 23.05 nm. NLE-AgNPs menunjukkan aktiviti antikulat yang sangat baik terhadap kedua-dua patogen berbahaya dalam ujian penyebaran sumuran agar dan ujian penyahaktifan kulat dalam air berbanding dengan rawatan menggunakan NLE sahaja. Mekanisme penyahaktifan dibuktikan berdasarkan analisis imej FESEM ke atas kulat yang tidak dirawat dan dirawat dari ujian penyahaktifan kulat dalam air. Imej kulat yang dirawat dengan NLE-AgNPs menunjukkan kerosakan struktur dan deformasi kulat dan boleh menyebabkan kerencatan kepada pertumbuhan kulat untuk pembiakan. NLE-AgNPs digunakan untuk merawat sampel air yang mengandungi kulat, dan penyahaktifan kulat telah dioptimumkan dengan menggunakan Kaedah Permukaan Rintangan (RSM) menggunakan perisian Design Expert (Stat-Ease Inc., Minneapolis, MN 55413, USA, versi 7.0.0). Kesan dos NLE-AgNPs (10-100 µg/L), pH (6-8), masa interaksi (40-240 min) serta kelajuan pengadukan (100 - 120 rpm) telah dikaji terhadap prestasi penyahaktifan kulat dalam RSM. Reka bentuk faktorial penuh (FFD) digunakan untuk menentukan dan menapis pemboleh ubah eksperimen yang mempengaruhi kecekapan penyahaktifan kulat. Saringan awal menunjukkan bahawa dos, masa interaksian, dan pH adalah faktor-faktor yang paling signifikan yang mempengaruhi proses penyahaktifan kulat dengan respons maksimum sebanyak 39.07%. Faktor-faktor signifikan ini telah dioptimumkan menggunakan Reka Bentuk Komposit Pusat (CCD) dan keadaan optimum bagi dos, pH, dan masa interaksi adalah masing-masing 105 µg/L, pH 6.5, dan 245 min, di mana respons pengasingan kulat mencapai maksimum sebanyak 51.03%. Nilai R^2 bagi proses penyahaktifan kulat menggunakan CCD adalah 0.9478, menunjukkan persetujuan yang baik antara data model eksperimen dan data ramalan. Hasil kajian ini mendapati ekstrak daun semambu dan derivatifnya, biosintesis NLE-AgNPs berpotensi untuk digunakan sebagai alternatif pembasmi kulat yang lebih mesra alam untuk digunakan dalam rawatan air.

ABSTRACT

Fungal contamination in the drinking water distribution system (DWDS) can generate taste and odor issues, ultimately affecting the quality of the delivered water. Thus, possibly causing a significant threat to public health. *Rhodotorula mucilaginosa* and *Aspergillus* sp. is one of the current emerging opportunistic waterborne pathogens isolated from DWDS that can colonize and infect immunocompromised individuals. The usage of chemical treatment to remove this pathogen may also leave disinfectant residuals, which are known for their toxicity, and rather, such an approach can be too expensive in some countries. This circumstance generated interest to develop an alternative disinfectant in water treatment which is more effective with less toxicity. This study attempted to use neem leaves extract (NLE) and environmentally friendly green synthesized silver nanoparticles mediated by neem leaves extract (NLE-AgNPs) as an alternative natural-based disinfectant or antifungal agent against both isolated fungi. The formation of NLE-AgNPs was confirmed through several characterization processes, including ultraviolet-visible spectroscopy (UV-Vis), Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), Field emission scanning electron microscope (FESEM), and Transmission electron microscope (TEM) analysis. The result revealed that the NLE-AgNPs showed UV-vis spectra peak around 421 nm, are crystalline in nature, uniformly well distributed and have a spherical morphology with an average size of 20.13 - 23.05 nm in diameter. NLE-AgNPs exhibit excellent antifungal activity against both waterborne pathogens on agar well diffusion assay and water disinfection test (fungal inactivation test) as compared to NLE alone. The inactivation mechanism was described based on the FESEM image analysis of untreated and treated fungi from the water disinfection test, which revealed structural damage and deformation of fungus when treated with NLE-AgNPs, causing retardation of fungus growth for further reproduction. The inactivation of fungi seeded in sample water treated with NLE-AgNPs was further optimized by response surface methodology (RSM) using the Design Expert software (Stat-Ease Inc., Minneapolis, MN 55413, USA, version 7.0.0). The effect of NLE-AgNPs dosage (10-100 µg/L), pH (6-8), contact time (40-240 min), as well as mixing speed (100–120 rpm) was studied on fungal inactivation performance in RSM. Full factorial design (FFD) was employed to determine and screen the experimental variables that significantly influence fungal inactivation efficiency. The initial screening indicated that dosage, contact time, and pH are the most significant factors affecting the fungal inactivation process, with a maximum response of 39.07%. These significant factors were optimized using Central Composite Design (CCD) and the optimum condition of the dosage, pH, and contact time were found to be 105 µg/L, pH 6.5, and 245 minutes, respectively at which the fungal inactivation response maximize up to 51.03%. R^2 for fungal inactivation process by using CCD results in a figure of 0.9478, indicating a good agreement between model experimental data and forecasting data. The results of this study provide insight into the potential of neem leaf extracts and their derivatives, biosynthesis NLE-AgNPs, as an alternative eco-friendly disinfectant and antifungal agents in water treatment.

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