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Green synthesis and characterisation of Zinc Oxide (ZnO) nanoparticles from two duckweeds of Malda District

[1]Sudipta Kumar Sil, *Santanu Gupta and [2]Moupriya Nag

[1]Department of Botany, University of Gour Banga, Malda- 732103 (India)
[*]Department of Botany, Malda College, Malda-732101 (India)
[2]Department of Biotechnology, University of Engineering and Management, New Town, Kolkata-700122 (India)
*Corresponding Author: Email : santanubotanist@gmail.com

Abstract

Duckweeds are aquatic angiosperms that proliferate luxuriously in tropical stagnant waters enriched in organics which serve as the sources of nitrogen and phosphorous to these small floating plants. In the district of Malda (W.B.), like other ecologically similar places, duckweeds form floating sheath over water surface. They are potential source of biomass used for green synthesis of nanoparticles like zinc oxide nanoparticles as carried out in this study. The existence of zinc oxide as nanoparticle was primarily understood through UV-spectrophotometry where absorption maxima recorded to have been 368 nm and 360 nm in case of *Spirodela polyrrhiza* (L.) Schleid and *Lemna minor* L. respectively as used as biomaterial. The shape and dimensions of these nanoparticles synthesized using *Lemna minor* L. and *Spirodela polyrrhiza* (L.) Schleid. were investigated by spectrophotometric and microscopic (SEM and TEM) methods.

Key words : Nanoparticles, Green synthesis, ZnO, Duckweeds, UV- vis Spectrophotometer, Scanning Electron Microscopy, Transmission Electron Microscope.

Stagnant water bodies like ponds and ditches are quite common in the tropical and subtropical countries like Bangladesh, Sri Lanka , India etc which forms suitable habitats for a lot of aquatic macrophytes.. Such waters in the ponds and ditches are used by inhabitants of Malda town for bathing , washing etc. that changes the physical and chemical parameters resulting in eutrophication[2]. In such eutrophic water bodies duckweeds show luxuriant grow. Duckweeds belong to the monocot order Alismatales, family Araceae that are represented by 5 genera namely *Lemna, Spirodela, Landoltia, Wollfia, & Wollfiella* consisting

[1]Professor, [*]Assistant Professor, [2]Assistant Professor.

of with 37 species all over the world[20]. On the surface of nutrient rich waters, duckweeds form thick mats and are capable of uptaking dissolved substances from such water bodies resulting enhancement in their biomass. Duckweeds are used for waste water treatment. Due to their ability to store nutrients above their metabolic need showing fast growth, and due to their fast growth rate , they are applicable for testing different mathematical models[19]. Species of *Lemna* and *Spirodela* produce transgenic proteins[15].

The lesser duckweeds, known as *Lemna minor* L. are cosmopolitan in occurrence. It bears a single long root with fronds having symmetrical or suborbicular shape. Reproduction takes place mainly by vegetative means, flowers rare, if produced are covered by spathe[7]. *Spirodela polyrrhiza* (L.) Schleid., are quite commonly found in the nutrient rich waters are also known as greater duckweeds[8]. It bears a flattened frond surface on both sides bearing 2-18 roots on the dorsal side. Unisexual flowers are produced inside covered lateral pouches. Under unfavourable conditions, turions are produced that sink to the bottom and on returns of favourable condition produces new fronds[5]. Formation of turion is also dependent on the level of phosphorus in the water bodies[1].

Nanoparticles synthesis has drawn attention recently owing to the material size. Nowadays green synthesis of metal nanoparticles is very interesting in the field of nanotechnology. Among the biological specimens, plant material is mostly preferred. Green synthesized nanoparticles from plants possess more stability, variation in the shape and size in comparison to the nanoparticles from other organisms[13]. Zinc Oxide (ZnO) nanoparticles are now widely studied because of its diversity in properties ranging from antimicrobial properties, industrial application and also for gene or drug delivery[10]. Owing to their high surface to volume ratio, concentration and uniqueness in their characteristics, they show remarkable antimicrobial property. Biologically synthesized nanoparticles are less toxic and ecofriendly in comparison to the chemically synthesized nanoparticles and reduces the threat to the environment[9]. There are different methods of ZnO nanoparticles such as reverse micelle, sol–gel method, homogenous precipitation, sonochemical method, hydrothermal method, microwave irradiation etc[6]. Milky latex of *Calotropis procera* and latex of *Aloe vera* were also found useful in the synthesis of ZnO nanoparticles[17]. Similar results were found using the latex of *Osimum basilicum* L. and *Parthenium hyterotropus* L[14]. Literature study suggests that successful synthesis of ZnO nanoparticles were achieved using orange juices[4]. ZnO nanoparticles are effective in very small amount against a wide range of pathogenic Gram positive and Gram negative bacteria as compared to chemically synthesized nanoparticles.

Malda district, once the capital of Bengal, is situated in the state West Bengal and covers about 3733 sq km and marks the entry point towards North Bengal. It is situated on banks of River Ganga with geographical location between 24°40' 20" N and 25°32'08" N latitude and 87°45'50' E and 88°25'10" E longitude with district headquarters in the English Bazzar of Malda Town city. The area

is topographically marked by low lying plains that roll in southward direction. The district is divided into the eastern part known as Rarh and western part known as Barind by the free flowing rain fed river Mahananda. The district comprises of two subdivisions with two municipalities namely English Bazar and Old Malda Water bodies represents 28.77% of the total area of Malda District. These water bodies are infested by a number of aquatic macrophytes which includes the duckweed species like *Lemna perpesuella* (L.) & *Spirodela polyrrhiza* (L.) Schleid[16]. The present paper focuses on the green synthesis of ZnO nanoparticles from two duckweeds namely *Lemna minor* L. and *Spirodela polyrrhiza* (L.) Schleid.

a. Collection of the plant extract :

Fresh plant materials of *Lemna minor* L. and *Spirodela polyrrhiza* (L.) Schleid. were collected from two water bodies located in the English Bazaar Municipality of Malda town in month of January 2022.

b. Preparation of the plant extract :

5gm fresh weight of each of the plant material (*Lemna minor* L. and *Spirodela polyrrhiza* (L.) Schleid) were weighed (only the fronds), washed under running tap water for 5 minutes followed by washing with double distilled water for 10 minutes each. Then both the plant materials were soaked in 100 ml of double distilled water taken in 250 ml conical flasks for 10 minutes. Then both the solutions were heated at 70°C for 8 minutes and then cooled up to room temperature. This was followed by filtering of both the plant extracts using Whatman No 1 filter paper and the filtrates were kept in the refrigerator for further experimental purpose.

c. Green synthesis of Zinc Oxide nanoparticles:

One gm of Zinc nitrate [$Zn(NO_3)_2$] hexahydrate was dissolved in 100 ml double distilled water and was kept in a magnetic stirrer for 1 hour. This solution was referred to as "Solution A." Another solution was prepared by dissolving 2gm sodium hydroxide (NaOH) in 25 ml of double distilled water, which is referred as "Solution B." After 20 minutes of preparation of "Solution B", it was mixed with "Solution A." To this mixture, 25 ml of plant extract was added and was kept in a magnetic stirrer. After 3hours, the colour of the solution was changed which confirmed the preliminary green synthesis of ZnO nanoparticles. It was followed by centrifugation of the solution at 6000 rpm for 15 min. The pellet after centrifugation was washed in HPLC graded distilled water and the supernatant was discarded. After that the pellet was stored in incubator at 80°C for SEM and TEM studies.

d. Characterization of Zinc Oxide nanoparticles :

The green synthesized ZnO NP was characterized with help of UV-Visible Spectrophotometer (Lasany Li295) with wavelength range of 300-500 nm and the spectra were recorded and analysed with its own software. The Scanning electron microscopy was performed in USIC, The University of North Bengal using JEOL (JSM-IT 100). It operated using Secondary Electron Detector of 5.0kV and working distance

10nm, Probe Current (PC-#30) and Accelarting Voltage 5.0kV. The Transmission Electron Microscopy of the green synthesized ZnO NP was performed in USIC, The University of Burdwan using JEM 1400 plus. The synthesized ZnO NP were suspended in methanol and dispersed uniformly in a sonicator. Very small amount of the dispersed ZnO NP was taken in a micropippete and 2-3 drops of it was loaded in a TEM grid of 400 mesh (Ted Pella).

Figure 1 represents the UV-vis spectra of newly synthesized ZnO nanoparticles using *Spirodela polyrrhiza* (L.) Schleid. The figure depicts that an absorption maxima at 368 nm which clearly demonstrates the green synthesis of ZnO NP in the reaction mixture. The absorption spectra also shows continuous synthesis of ZnO NP in the reaction mixture. Figure 2 shows the UV-Vis absorption spectra of newly synthesized ZnO nanoparticles using *L. minor* L. leaf extract. The figure shows absorption maxima at 360 nm confirming green synthesis of ZnO NP in the reaction mixture. The absorption spectra also shows continuous green synthesis of ZnO NP in the reaction mixture. UV –visible absorption spectrum gives us conclusive evidence regarding the shape and size of green synthesized of ZnO nanoparticles in aqueous solution[11]. The exited electron starts oscillating due surface plasmon resonance effect and particular wavelength which are detected through UV- visible spectroscopy[12]. Colour change of the solution to light green gives confirmation regarding green synthesis of ZnO NPs[18]. ZnO NP synthesized in the aqueous medium exhibit maximum UV- vis absorption between 360 to 375 nm due to maximum excitement of electrons[3].

Further characterization of green synthesized ZnO NPs using *Spirodela polyrrhiza* L. Schleid. and *Lemna minor* L. were performed using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) as shown in figure 3 (A to D). SEM studies showed that the green

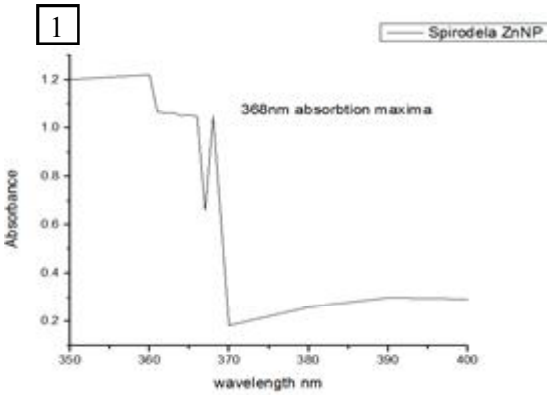

Figure1- Absorption spectra of ZnO Nanoparticles from *Spirodela polyrrhiza* (L.) Schleid.

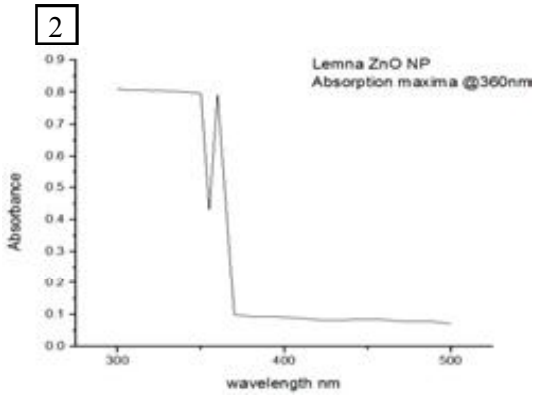

Figure 2-Absorption spectra of Green synthesized ZnO Nanoparticles of *Lemna minor*. L.

synthesized ZnO NP ranges between 0.025 µm to 0.040 µm in case of *Spirodela polyrrhiza* L.Schleid. and 0.045 µm to 0.060 µm in case of *Lemna minor* L. TEM gave further insight into the green synthesized nanoparticles. Shape of the green synthesized ZnO nano particles using biomas of both the duckweeds were found to be ovoid in shape as revealed in TEM (Figure 3 C &D).

So the results of the experiment indicates that nanoparticles synthesized using biomass of *Spirodela polyrrhiza* L. Schleid and *Lemna minor* L. ranges between 0.025 µm to 0.060 µm and ovoid to spherical in shape. These nanoparticles can be assessed for their anti microbial efficacy against pathogenic microorganisms.

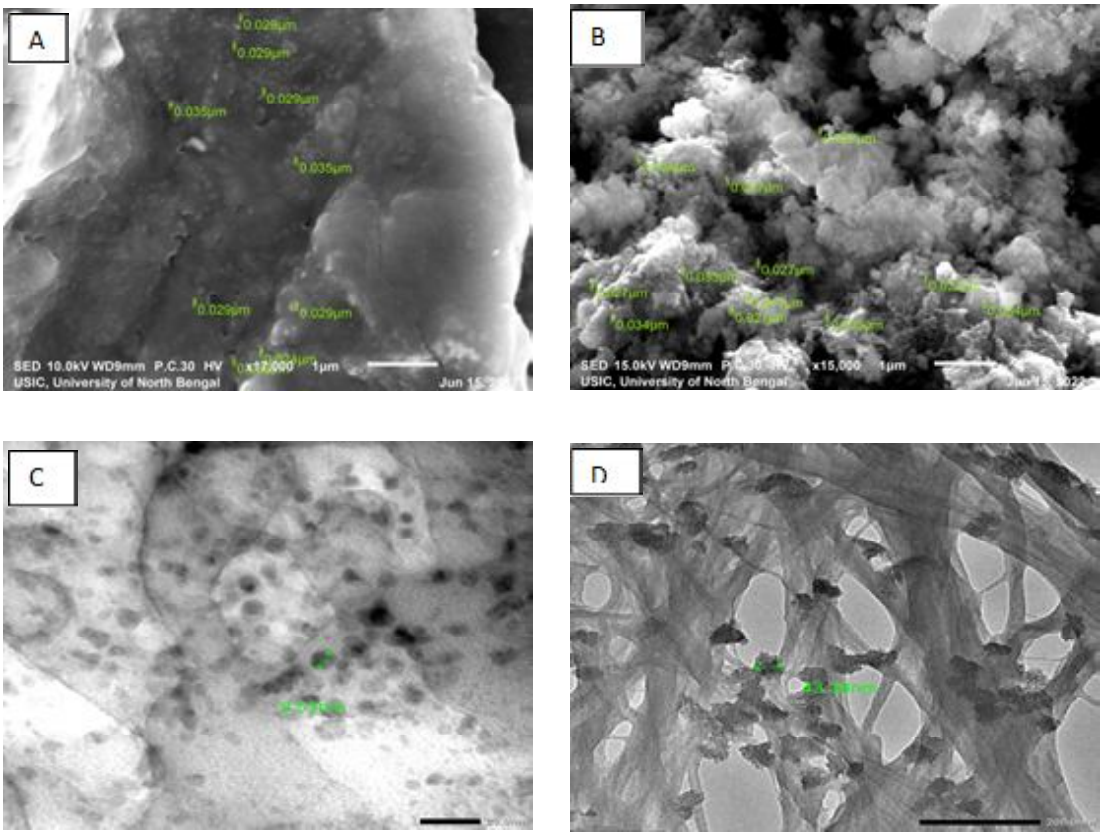

Figure3 – Electron micrographs of *Spirodela polyrrhiza* (L.) Schleid. and *Lemna minor* L.

A- Scanning electron micrograph of *Spirodela polyrrhiza* (L.) Schleid.

B- Scanning electron micrograph of *Lemna minor* L.

C- Transmission electron micrograph of *Spirodela polyrrhiza* (L.) Schleid.

D- Transmission electron micrograph of *Lemna minor* L.

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