Journal of Basic and Environmental Sciences

ISSN
Online:2356-6388
Print:2536-9202

Research Paper Open Access

Myco-synthesis of silver and ZnO nanomaterials using endophytic fungi isolated from different locations in Egypt for sustainable development

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Abstract:

The approach of biosynthesis of nanoparticles utilizing endophytic fungus is very promising due to its efficacy, environmental friendliness, cost-effectiveness, and straightforward procedures. In this work, we examined six endophytic fungal isolates derived from various plants to determine their ability to produce four types of nanoparticles: AgNPs, CuNPs, SiONPs, and ZnONPs. Preliminary identification of nanoparticle synthesis using UV-Visible spectrophotometer and particle size analyzer revealed the presence of two distinct types of nanoparticles: ZnONPs and AgNPs. The isolated fungus TRA2 produced silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnONPs) with average diameters of 18 and 28.7 nm, respectively. Comparatively, the fungus TRC1 produced silver nanoparticles (AgNPs) with average diameters of 22.8 nm and zinc oxide nanoparticles (ZnONPs) with average diameters of 73.8 nm. Morphological identification of the two isolates (TRA2 and TRC1) revealed their respective species as *Talaromyces sp*. and *Chaetomium sp*. The identified isolates show great potential as viable options for the production of bionanomaterials aimed to achieve sustainable development

Key words: *Nanomaterial, Silver-nanoparticle, Zincoxide-nanoparticle, Talaromyces sp., Chaetomium sp.*

1. Introduction

Nanotechnology has become a fundamental technology that has completely transformed all areas of applied science. Nanoparticles (NPs) are a subset of nanotechnology that comprises nanoscale materials with unique characteristics due to their very small size and high surface area to volume ratio. These features have resulted in notable differences in properties compared to their bulk counterparts **[1]**.

The relevance of nanotechnology applications in the agricultural sector has emerged only in recent years, while the research in this field was initiated around fifty years ago **[2].** The utilization of nanomaterials is necessary to enhance the efficiency of fertilization use, increase yields, decrease the need for pesticides, enable rapid and early detection of pathogens and toxic chemicals in food products, develop intelligent systems for delivering pesticides and fertilizers, improve food packaging and processing, and regulate agricultural food security **[3].**

Nanoparticles are traditionally synthesised using physical and chemical techniques, which offer a higher production rate and allow for more accurate size regulation of NPs. However, these methods are considered unfavorable due to their substantial initial investment burden, significant energy requirements, and use of toxic and hazardous substances. Furthermore, the process of chemically producing nanoparticles is poisonous and less suitable with living creatures **[4]**.

Recently, the environmentally friendly synthesis of nanoparticles (NPs) has emerged as a feasible substitute for conventional physical and chemical methods by employing biological-mediated approaches. Biochemical synthesis of metal and metal oxide nanoparticles (NPs) is facilitated by monocellular and multicellular biological organisms, including bacteria **[5]**, yeast **[6]**, fungus **[7]**, and other agents. The aforementioned methods are economically efficient, non-hazardous, and ecologically viable. Microbial organisms behave as a tiny nano-factory, where enzymes and other biomolecule components, either released or synthesized by the bacteria, reduce metal ions into metal nanoparticles (NPs).

The objective of this work is to extract endophytic fungus from several host plants and evaluate their capacity to generate Nanomaterials. These nanoparticles are then identified using a UV-visible spectrophotometer and particle size instrument. A graphical abstract of the present work is shown in **Figure 1**.

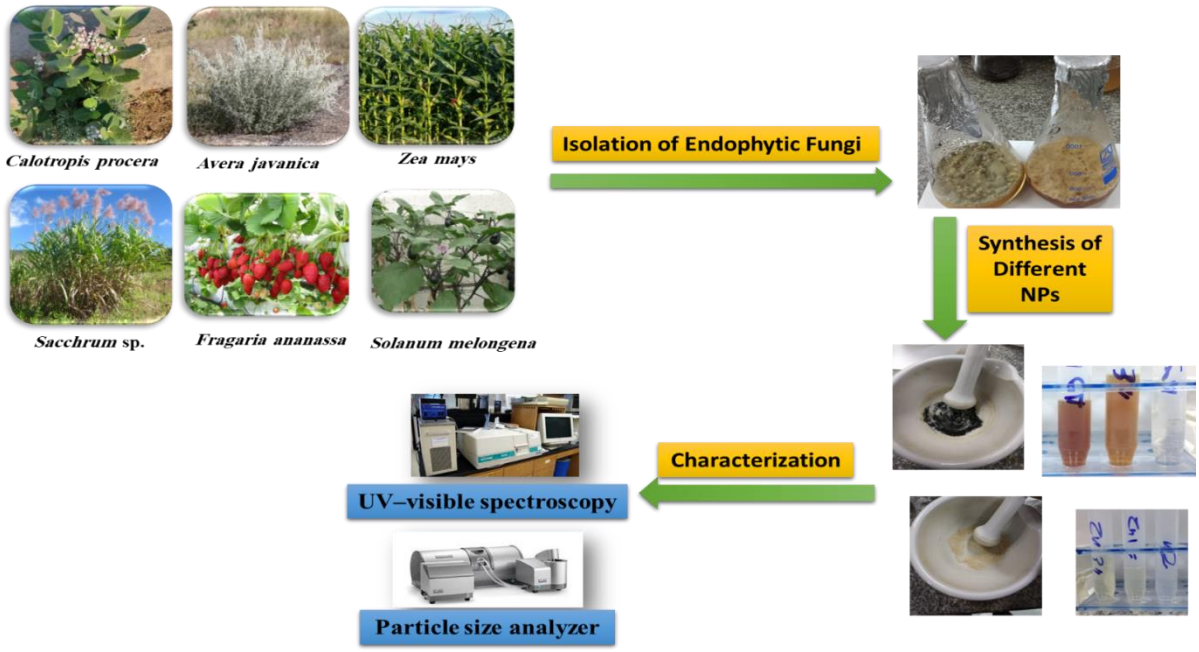

Fig. (1): Graphical Abstract for the current study.

2. Materials and Methods

2.1. Isolation of Endophytic fungi

Individual plants from various sites were selected at random, placed into plastic bags, and stored on ice to isolate possible endophytic fungus. Surface sterilization of plant roots was achieved by submerging them in a 70% ethanol solution for three minutes, followed by immersion in a 2-4% aqueous solution of sodium hypochlorite for five minutes, and then immersing them in 70% ethanol for one minute. Afterwards, the materials were rinsed twice in sterile distilled water and then dried in sterilized paper under a laminar flow hood to eliminate any surface sterilization agents. The sterilized plant roots were pulverized in a sterile environment, and the remaining liquids were placed into potato dextrose agar (PDA) medium and left to incubate at 28°C for 72 hours. Following incubation, every colony isolated from the sections was transferred to antibiotic-free potato dextrose agar medium (PDA) for further examination **[8]**.

2.2. Preparation of fungal biomass

The fungal isolates were introduced into Potato Dextrose broth medium and subjected to fermentation at a temperature of 30± 2 °C for a duration of five days using an orbital shaker operating at 120 rpm. Subsequently, the mycelium of each fungal isolate was collected by separation using a 0.45 µm membrane filter, rinsed three times with double distilled sterile water to remove any remaining traces of broth medium, and then dried at a temperature of 50 °C.

2.3. Biosynthesis of Nanoparticles

Each fungal isolate was inoculated into 250 mL Erlenmeyer flasks containing 100 mM of various salts as the precursor for nanoparticle synthesis. The flasks were then incubated under shaking at 150 rpm for 72 hours at 28±2 °C. The synthesis of AgNPs, CuNPs, SiONPs, and ZnONPs was carried out using the following salts: AgNO4, CuSO4, Na2SiF4, and ZnSO4, respectively. The particles produced were purified by filtering with a sterile Millipore 0.22 µm syringe filter, then subjected to dialysis and centrifugation, and forwarded for further characterization. The appearance of a reddish brown hue in the colloidal solution showed the presence of AgNPs; however, the solution containing ZnO NPs or SiONPs appeared white. Furthermore, the produced CuNPs caused a transformation of the $CuSO_4$ solution from a light blue hue to a deep green shade. Consequently, the nanoparticles were gathered by centrifugation at 18,000×g for 10 minutes and then rinsed with distilled water and ethanol to eliminate any residual salts from the reaction mixture before being centrifuged **[9]**.

2.4. Detection of the synthesis of different Nanoparticles

Initial verification of several nanoparticle designs was conducted using a UV-Visible spectrophotometer (**Jenway, Model 6715 UV/VIS**). One milliliter of the samples was placed in each well of a two-well plate, and the scanning procedure was performed in triplicate at 25 degrees Celsius, covering a wavelength range of 190 to 800 nm. Specific samples were selected for further studies in which Nanoparticles were examined using UV-Vis spectroscopy. The mean diameter was determined using a particle size analyzer (NICOMP N3000-model).

2.5. Identification of fungal isolates

The fungal isolates that produced nanoparticles were purified and preserved on the same medium (PDA) and morphologically characterized using a microscope, following the standard procedure outlined by **[10]** at the Nano-Phytopathology Lab. (NPPL), Desert Research Center, Cairo, Egypt. For further investigations, the acquired isolates were subcultured on PDA slants and refrigerated at 4°C.

3. Results:

3.1. Isolation and identification of endophytic fungal isolates

The results presented in Table 1 show that six endophytic fungal isolates were collected from various plants in two locations in Egypt, namely the 10th of Ramadan and Shibin El Qanater. The fungi obtained from the 10th of Ramadan

were designated as TR, while those obtained from Shibin El Qanater were designated as Sh. Two fungal isolates were collected on the 10th of Ramadan, while four fungal isolates were retrieved from Shibin Shibin El Qanater. This study examined the isolates for their ability to produce four types of nanoparticles: AgNPs, CuNPs, SiONPs, and ZnONPs.

Table (1): Endophytic fungal isolates from different locations.

No.	Sites	Host plant	Fungal code
1	**10th of Ramadan**	*Calotropis procera*	TRC1
2		*Avera javanica*	TRA2
3	**Shibin El Qanater**	*Zea mays*	ShZ3
4		*Sacchrum sp.*	ShS5
5		*Fragaria ananassa*	ShF6
6		*Solanum melongena*	ShS8

3.2. Detection of the synthesis of different Nanoparticles

Nano particles and their precursor were subjected to the UV spectra to confirm the conversion of zinc nitrate into zinc oxide. Zinc nitrate showed absorption at 300 nm, whereas prepared ZnO at 370 nm. A very low peak of bulk ZnO was observed at 388nm.As the peak of bulk Zinc Oxide has been shifted to 370 nm which clearly evident the preparation of nano ZnO as shown in **Figure 1.**

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UV-visible spectroscopy is the frequently employed method for studying the optical characteristics of particles. The sixth fungal isolates were analyzed to determine their capacity to produce four different types of Nanoparticles. The resulting samples were then analyzed

using UV spectra to verify the creation of nanobiomaterials. The UV-Vis photoluminescence spectra of many nanoparticles is presented in **Figure 2**.

Within each sample, two peaks were seen for AgNPs. However, only two fungal isolates (TRC1 and TRA2) showed evidence of AgNPs production. A single peak was identified in the ultraviolet (UV) area at wavelengths of 220-248 nm, along with additional wide bands in the visible range at 338.5 – 384 nm, as shown in **Figure 2 (A&B)**. The detection of a wide absorbance peak at 428 nm in the Ag/Ago sample was reported by **[11]** and **[12]**. This peak is attributed to the presence of Ag nanoparticles. A study conducted by **[13]** revealed that the change in absorbance spectra and surface plasmon resonance (SPR) of external Ag NPs towards shorter wavelengths, as opposed to intracellular Ag NPs, may be ascribed to the decrease in the particle size of AgNP.

Similar patterns were seen in the samples of ZnONPs. Zinc salt exhibited absorption at wide peaks between 213 and 260 nm, but the synthesized ZnONPs had a broad peak range from 333 to 368 nm, as depicted in **Figure 2 (C&D).** Furthermore, this phenomenon is exclusively seen in the two fungal isolates TRC1 and TRA2. The adjustment of the peak of bulk Zinc Oxide to 370 nm clearly indicates the synthesis of nano ZnO **[14]**. The zinc sulfate heptahydrate crystal exhibits transparency within the ultraviolet (UV) spectrum **[15]**.

In relation to SiO2NPs, as shown in **Figure 2 (E&F)**, the UV-VIS spectra exhibited their characteristic peaks: a distinct peak in the ultraviolet (UV) range at 200-218 nm and a broad band in the visible range at 353-373 nm, which signifies the presence of silicon dioxide nanoflakes. The visible range bands were exclusively observed in the two fungal isolates identified as TRC1 and TRA2, indicating that only these isolates were capable of synthesizing the SiO2NPs. The results we obtained align with those of **[16]**, who established that the SiO2 salts photocatalyst exhibits significant absorption in the ultraviolet (UV) region, with the absorbance measured at 297 nm.

Regarding CuNPs, the six fungal isolates exhibited just one peak at 230-270 nm. No peaks were seen in the visible region, indicating that none of the six fungal isolates were able to produce CuNPs. In contrast, **[13]** discovered that CuNPs exhibited surface plasmon resonance (SPR) at the wavelength range of 562–573 nm.

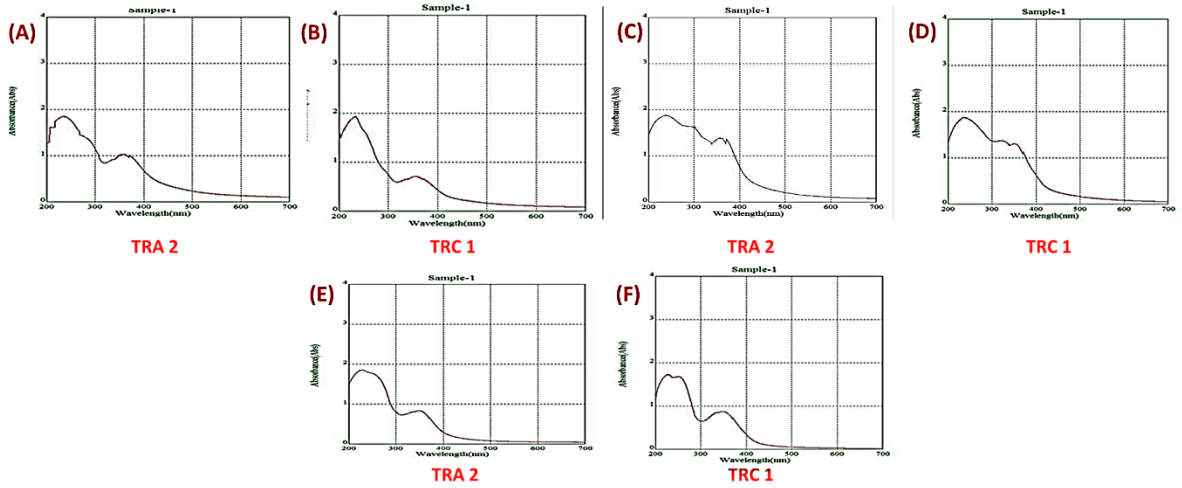

Fig. (2): UV-Visible spectra of Ag NPs (A&B), SiO2 NPs (C&D), ZnONPs (E&F) of two fungal isolates TRA2 and TRC1

3.3. Particle size analyzer:

The particle size measurement showed that only silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnONPs) were verified to be produced by the two fungal isolates, as shown in **Figure 2**. The average diameter recorded for the fungus TRA2 was 18 nm for AgNPs, 820.4 nm for SiO_2NPs, and 28.7 nm for ZnNPs, as seen in **Figure 3 (A, B & C)**.

Regarding the fungus TRC1, the average diameters were 22.8 nm for AgNPs, 1039.9 nm for SiO2NPs, and 73.8 nm for ZnNPs **(Fig.3. (D,E&F))**, indicating its existence within the nano-materials range reaching 100 nm. These findings indicate the existence of only silver nanoparticles (AgNPs) and zinc nanoparticles (ZnNPs) within the nano-materials range of 100 nm, as reported by **[17]**.

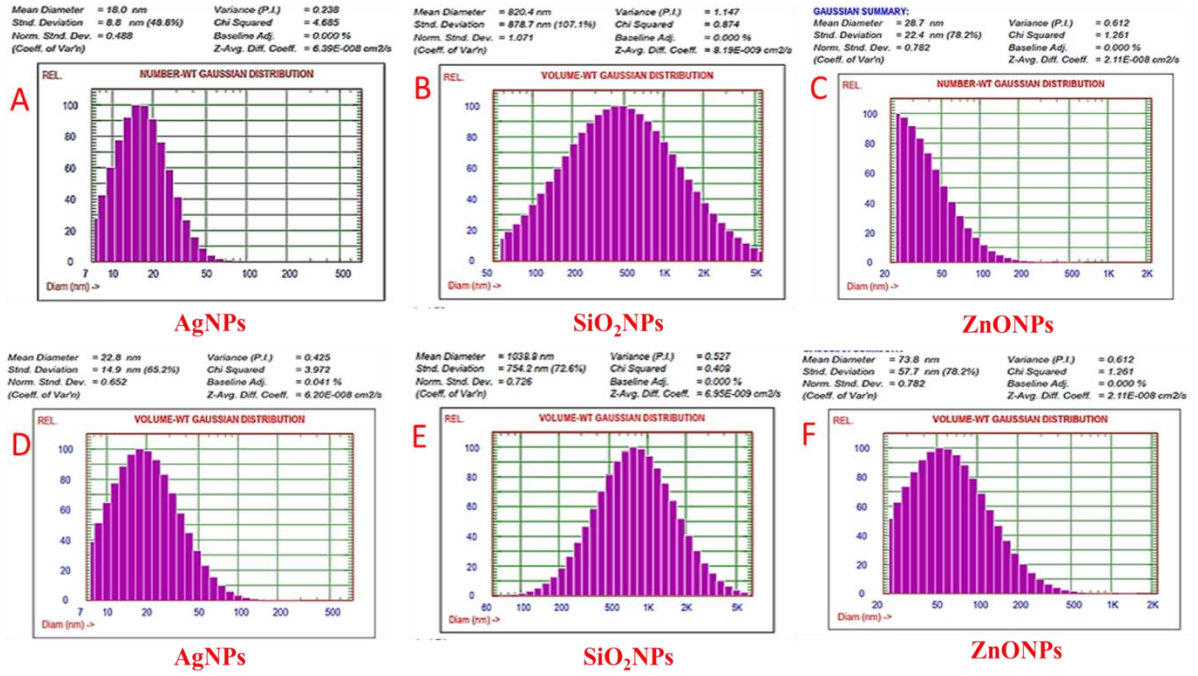

Fig. (3): A, B, C: Particle Size of Nanoparticles synthesized by fungus coded TRA2, D,E,F: Particle Size Of Nanoparticles synthesized by fungus coded TRC1.

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3.4. Identification of fungal isolates

Based on the morphological identification of two fungal isolates, *Talaromyces sp.* was recognized as the fungus coded TRA2, whereas *Chaetomium sp.* was identified as the fungus coded TRC1.

Ultimately, the fungal isolates found in *Talaromyces sp.* and *Chaetomium sp.* show great potential as suppliers for the synthesis of ZnO NPs and AgNPs. This environmentally friendly synthesised bio nanoparticles are highly suggested for the purpose of sustainable development.

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