# Green synthesis of silver nanoparticles by cyanobacterial extracts: an approach guarantees potential bioactivity and proper cereal seed germination

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**Background**
The last few decades witnessed the adoption of green nanotechnology as an environmentally friendly and cost-effective strategy with several biomedical, industrial and agricultural applications. Here, cyanobacteria have been suggested as model microorganisms for bio-nanoparticles production.
**Objective**
In the present study, the extracts of a number of cyanobacterial isolates representing different genera and isolated from various aquatic environments of Egypt were explored as a novel source of bioactive silver-based nano-materials.
**Materials and methods**
The synthesized nanoparticles were characterized by UV-visible spectroscopy (UV-Vis), transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy (FTIR). Results revealed the presence of secondary metabolites in the aqueous extracts necessary for the nano-material biosynthesis. The biological potentials of the produced crude extract-based silver nanoparticles (AgNPs) assessed as antimicrobials indicated high activities against several bacterial and fungal pathogens. These bioactive nanoproducts exhibited antioxidant effects as well.
**Results and conclusion**
When extract-based AgNPs were tested for seed germination and seedling development of barley (cvs. Giza-123, Giza-2000) and wheat (cvs. Benisweif-7, Misr-3), relative increases in the germination percentages, germination rate index (GRI%), germination velocity coefficient (GVC%) were scored together with somewhat reductions in the mean germination times (MGT). All in all, the findings of this work emphasis that such silver nanoparticles possess antimicrobial and antioxidant activities besides supporting seed germination and seedling development, hence they are highly recommended as an alternative to high-risk chemically synthetic agrochemicals with no expected phytotoxicity.

**Keywords:**
cyanobacteria, green synthesis, silver nanoparticles, antimicrobial, antioxidant, seed germination

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## Introduction

Actually, a vast array of antibiotics commonly used today for treating infectious microorganisms is becoming ineffective as a result of emergence and spreading of antibiotic-resistant microbial strains. That is why researchers recently focused on finding untraditional new agents that have potential antibiosis towards pathogenic microorganisms. In this context, nanoparticles are being considered as a good alternative candidate in a variety of cases.

Nanotechnology, nowadays, is an emerging field of research that encompass the synthesis, characterization and the development of different nano-materials that having prominent roles in everyday life. The term 'nano' refers to a particle or material characterized by a very small size of at least one nano-scale. As mentioned by Jeon *et al* [1], nanoparticles possess unique and novel properties when compared with larger ones. Of those properties, biochemical reactivity, magnetism, electrical conductivity, optical impacts besides physical strength are well established [2]. The major reason for the superiority of nano-materials over the larger ones is due to their very small sizes [3]. Thousands of synthetic and commercial products of nano-structures are expected to become a global business in the coming years.

Along years ago, silver is well known for its medicinal properties where it possesses broad-spectrum biological potentials. Keeping in view, silver nano-particles (Si-NPs) have substantial optical and thermal properties, catalytic activity, and electrical conductivity in addition to chemical stability and antimicrobial activity [4]. Such particular advantages did support the introduction of Si-NPs for several applications as anticoagulants, antimicrobials, anticancer, drug delivery, orthopedics, medical devices sensing and diagnostics and others in various fields [5]. The released silver ions ($Ag^+$) from Si-based nanoparticles proved to play a crucial role in the antibiosis towards living micro-creatures [6]. The antagonistic potential of silver nanoparticles (AgNPs) is mainly attributed to their combined influence of binding the $Ag^+$ ions to microbial cell walls, suppressing the membrane-associated enzymes, accumulation in the internal tissues, interference with the existing biomolecules of the cell, denaturation of cell envelope in addition to formation of reactive oxygen species. All of these events were reported to have detrimental effects on the microbial cells [7,8]. Multiple studies documented that AgNPs had obvious event even at quite low concentrations against methicillin-resistant *Staphylococcus aureus* (MRSA) strains, where the nano-material caused disruption of the bacterial cell wall [9,10]. Furthermore, AgNPs are commonly used in the electric and electronic fields due to their exceptional stability, conductivity beside high performance. As well, Si-based nanoparticles are in great demand in the textile industries as well as in food and beverage industries for food storage and packing processes [11]. This is beside their successful application for both detection and treatment of cancer [12].

Plenty of studies revealed significant effects of AgNPs on plant seed attributes including germination index, germination potential, mean germination time, seed vigor index, in addition to fresh and dry weights of developed seedlings [13–15]. Tariq *et al* [16]. mentioned that nano-materials, in general, are protecting plants from any adverse factor in two ways; as protection particles, and/or as carriers for the used pesticides and possibly other actives like double-strand RNA.

Conventionally, silver-based nanoparticles could be synthesized adopting different physical, chemical and biological or green methods. Physical procedures include vapor condensation, arc discharge and laser ablation [17]. Chemical strategies, on the other hand, encompass reduction and microemulsion methods as well as polyol process [18,19]. Unfortunately, the majority of these methods are quite expensive, time consuming, require high temperature, pressure and energy beside generating and consuming hazardous products that posing great threats to both environment and life forms [20,21]. Here, the drawbacks associated with the previously mentioned methods did necessitate the urgent need for developing non-toxic and environment-friendly manners to guarantee the safe synthesis of silver-based nanoparticles.

For positive deal with this issue, biologically inspired way is evolved to repair the disadvantages of non-biological methods applied for nanoparticles synthesis. As an alternative option, different biological sources of both prokaryotic and eukaryotic ranging from microorganisms to higher plants have been used to employ biosynthesis of metal nanoparticles (green synthesis). This way of synthesis involves using biological agents, *e.g.,* plant extracts, bacteria, fungi and algae. Furthermore, numerous studies approved the significant use of some natural polymers such as chitosan, soluble starch, polypeptide, heparin in addition to hyaluronan as capping and reducing agents for green synthesis of nano-materials [22].

Among the beneficial and environmentally friendly microorganisms recently used for green biosynthesis of nanoparticles, cyanobacteria and algae are of special concern. This refers to their capability to take in metals and reduce metal ions, low production costs besides easily production of nanoparticles on a large scale [23,24]. Extra interesting trait is their conspicuous tolerance to rigorous atmospheric conditions more efficiently compared to other microbiota. Both living and non-living dry biomass of algae are possibly used for nanoparticles biosynthesis, and therefore they usually known as 'Bionanofactories' [25].

Cyanobacteria are known as primitive and photosynthetic creatures of both ecological and economical importance. They are able to survive and grow in various extreme environments. They possess extraordinary traits of oxygen photosynthesis, biological dinitrogen fixation, high biomass production and habitat diversity. Additionally, they are having great capability to grow on non-arable lands, production of useful metabolites, generation of biofuel, in addition to improving soil fertility and reducing greenhouse gas emissions [26]. Many cyanobacterial species have been successfully used for nanoparticle synthesis including *Aphanocapsa,*

*Aphanothece, Calothrix, Cylindrospermum, Gloeocapsa, Leptolyngbya, Lyngbya, Nostoc, Oscillatoria, Phormidium, Spirulina* and *Synechococcus* [27].

For full utilizing the advantages of nanotechnology in the field of disease protection and plant growth promotion, it was necessary in the present study to investigate the biosynthesis and characterization of silver-based nanoparticles produced by some native cyanobacterial species secured from different aquatic environments of Egypt. Their extracts were explored as potential source of biologically active agents towards a number of harmful enemies as well as their possible beneficial impact on seed germination and seedling development.

## Materials and methods

### Water samples collection

Water samples were collected from different environments including Nile River, Mediterranean Sea, Red Sea, Ras El-Bar estuary, Karoun's Lake, Khadra Lake (salt marshes) and artificial freshwater pond located at Faculty of Agriculture, Cairo University (Table 1). Samples were enriched on Allen and Arnon with and without nitrates [28], ASN III, BG 11 and BG $11_0$ [29] liquid media. Inoculated flasks were incubated at 25°C for 7 to 30 days under continuous illumination (1000 lux) followed by purification techniques. All samples were transferred in an ice box at 4°C and examined one day after.

### Isolation of cyanobacteria

For isolation of representative cyanobacterial candidates, the procedure recommended by Rippka [30] was used. The liquid enrichment cultures were prepared from different water samples using the suitable medium of either. Those media are Allen and Arnon, Allen and Arnon plus nitrate, $BG11_0$ and BG11 for fresh waters. ASNIII, $BG11_0$ and BG11 for marine waters. ASNIII, Allen and Arnon, Allen and Arnon plus nitrate, $BG11_0$ and BG11 media for brackish waters. Ten ml of water sample were aseptically added as inoculum to 100 ml liquid medium in a conical flask (250 ml). Inoculated flasks were incubated at 25°C for 7 to 30 days under continuous illumination with Philips fluorescent white lamps, at a relatively low light intensity of 1000 lux.

### Purification of isolated cyanobacteria

The axenic cultures of the experimented cyanobacteria were obtained adopting the following techniques:

#### *Several successive transfers*

The isolated cyanobacteria, which had been previously enriched were successively subcultured several times on

**Table 1 Ecological profile of collected water samples**

| Governorate | Sampling site | Latitude/longitude | Sample nature/ Code |
|---|---|---|---|
| Giza | River Nile | 30°01'57.7'N<br>31°13'10.9'E | Fresh/ GiN |
| | Artificial pond (Fac. of Agric., Cairo Univ.) | 30°01'09.3'N<br>31°12'38.6'E | Fresh/GiAP |
| Damietta | River Nile | 31°27'53.7'N<br>31°49'16.2'E | Fresh/DaN |
| | Mediterranean Sea | 31°30'34.2'N<br>31°48'51.2'E | Marine/DaM |
| | Ras El Bar estuary | 31°31'37.4'N<br>31°50'38.7'E | Brackish/DaRB |
| Alexandria | Mediterranean Sea (Sedi Krerr) | 30°59'38.0'N<br>29°33'54.9'E | Marine/AlM |
| Red Sea | Ras Ghareb | 28°22'19.2'N<br>33°04'55.4'E | Marine/RSRGh |
| Suez | Ain Sokhna | 29°47'17.8'N<br>32°26'25.7'E | Marine/SuAS |
| South Sinai | Sharm El Sheikh | 27°57'34.7'N<br>34°23'54.2'E | Marine/SSSh |
| | Dahab | 28°29'31.4'N<br>34°31'02.7'E | Marine/SSD |
| Fayoum | Karoun's Lake | 29°28'06.8'N<br>30°45'38.8'E | Marine/FaK |
| Beheira | Wadi El-Natron, Khadra Lake | 30°26'31.3'N<br>30°13'37.3'E | Brackish/BeW |

the same culture media and incubated for 3–4 weeks at 25°C until healthy and homogenous cultures were obtained [30].

*Streak plate method*

After several successive transfers, the cyanobacterial cultures were streaked on their appropriate agar media to obtain single cyanobacterial colonies. Plates were sealed using Parafilm and incubated for 7 to 10 days at 25°C under continuous illumination (1000 Lux). This technique is preferable with unicellular and fast-growing cyanobacteria [30].

*Uni-filament isolation*

This technique depends on shedding unidirectional source of light on cyanobacterial agar plate, gliding movement and phototaxis of the microorganisms. The non-axenic cultures were grown on their proper agar media in Petri dishes to examine the ability of the filaments to grow and glide towards a single source of light to pick a single filament of cyanobacteria. Once the single filament moved a sufficient distance, it was removed under sterilized conditions and placed into a separate flask containing selective liquid culture medium, this technique is preferable with filamentous cyanobacteria [30,31].

Apart from the implemented isolation procedure, all pure isolates were maintained under photoautotrophic growth conditions in their selective media for further antibiotic purification to exclude the associated heterotrophic bacteria [32].

*Antibiotic purification*

The secured cultures were further purified to obtain axenic cultures. Isolates were grown on their recommended culture media supplemented with cycloserine antibiotic to avoid other heterotrophic bacteria possibly existing in association with the target cyanobacteria [32].

## Identification of cyanobacterial isolates

The total 11 obtained cyanobacterial candidates representing the three water types (five fresh, three marine and three brackish) were subjected to morphological features based on the procedures described by Rippka *et al.* and Castenholz [29,33]. Those traits included morphotype nature, size, shape of vegetative cells, presence of heterocyst and akinetes, this was followed by photographing using light microscope.

## Biosynthesis of silver nanoparticles (AgNPs) based on cyanobacterial extracts

The aqueous extracts of tested cyanobacterial isolates were assessed for their capabilities to deliver silver nanoparticles (AgNPs) under different production conditions of $AgNO_3$ concentration (0.5,1 and 2 mM), temperature range of 20–60°C, pH values of 4.5, 7 and 8.5 as well as illumination. The cyanobacterial biomasses were collected after reaching their log phase and centrifuged at 4000 rpm for 10 min at 20°C. The supernatants discarded and the pellets were washed 3 times with sterile distilled water. The pellets were added to 10 ml sterile distilled water and sonicated for 15 min at 100 amplitude using Ultrasonic Homogenizer (Cole-Parmer Instrumental Co. Chicago, Illinois 66648 USA). The obtained homogenates were filtered through Whatman No. 1 filter paper to remove cell debris followed by bacterial filtration using EZFlow® 33 mm Sterile Syringe Filter, 0.45 µm Hydrophilic Polyvinylidene Fluoride (PVDF). Then filtrates were aseptically completed to 100 ml with sterile distilled water and stored at 4°C. The dry weights of the biomass were determined.

For biosynthesis of AgNPs, 5 ml of the prepared aqueous extracts were completed to 19 ml by 0.1 M phosphate buffer at pHs 4.5, 7 and 8.5, then 1 ml silver nitrate solutions (0.5, 1.0 and 2.0 mM) were added separately for each pH level. The reaction mixtures were incubated @20, 30, 40, 50 and 60±2°C under both illumination and dark conditions.

The initial mixture was colorless or pale green. Production of AgNPs was monitored by formation of a brownish-yellow color of the $AgNO_3$ aqueous solution due to the excitation of the surface plasmon resonance (SPR) [34,35].

The biosynthesized AgNPs were collected by centrifugation (Centurion Scientific Pro-Research. HK241 United Kingdom) at 15 000 rpm for 10 min, washed with deionized water, dried and stored at ambient temperature.

## Characterization of crude extract- based AgNPs

### Ultraviolet-visible (UV–Vis) spectroscopic photometry

The change in color of the reaction mixture was observed by naked eye, where reduction of silver nitrate was recorded using the UV-visible spectrophotometer in the range of 200–700 nm with a resolution of 1 nm (Thermo Scientific Helios gamma, spectrophotometer, England) after 72 hours for

scanning the produced AgNPs. Samples showing absorption peaks in the range 400–450 nm were considered as silver nanoparticles as mentioned by Forough and Farhadi [36], while $AgNO_3$ solution was used as a control.

#### Transmission electron microscopy (TEM)

The AgNPs morphologies were observed by two-dimensional high-resolution AgNPs images captured using TEM (Jeol, JEM-1400, Japan) at an operating voltage of 80 kV. A drop of AgNPs suspension was placed on copper-grid carbon coated with 300 mesh palladium and carbon and allowed to dry. Both size and shape of the produced nano-materials were photographed.

#### Fourier transform infrared spectroscopy (FTIR)

The functional groups attached to AgNPs surface were identified with an FTIR spectrometer (Nicolet iS50). The dried nanoparticles mixture with potassium bromide in a ratio of 1:100 was prepared. A sample of 100 µl was placed in the attenuated total reflectance analyzer. The silver nanoparticle solution was analyzed by ATR-FTIR. The IR-rays spectrum was scanned across 4000-400/cm, with diffuse reflectance mode (DRS-800) within 4 $cm^{-1}$ resolution [37] (https://www.IR Spectrum Table and Chart, Sigma-Aldrich).

## Biological potentials of silver nanoparticles

The biological activities of tested cyanobacterial AgNPs assessed in the present study were limited to antimicrobials and antioxidants.

#### Antimicrobial activity

The antibacterial activities of the extract-based AgNPs were experimented adopting the well diffusion assay [38]. Four bacterial strains, obtained from the culture collection of the Cairo University Research Park, (CURP), were tested. Those are *Bacillus cereus* ATCC 33018, *Escherichia coli* O157:H7 wild type strain 93111, *Salmonella enterica* sub sp. *enterica* serovar *typhimurium* ATCC 14028 and *Staphylococcus aureus* sub sp. *aureus* ATCC 29213. As well, the antifungal potentials of the experimented materials were assessed against *Aspergillus niger* NRRL 326 and *Candida albicans* ATCC 10231. Broth cultures of bacterial strains were prepared in nutrient broth [39] with incubation at 30°C for 24 h., while fungal strains were cultivated in the same medium supplemented with 20 g/L glucose [40] and incubated at 30°C for 48 h. Each culture was spread on the surface of nutrient agar plates using sterile cell spreader, in which the wells of 8 mm diameter were prepared using a sterile cork borer. Either aqueous extracts or their AgNPs-based solutions were added into wells as the main treatments. Other sets of wells were filled with 50 µl of doxycycline (50 mg/ml) used as positive control for bacteria, while 50 µl fluconazole (100 mg/ml) was used as positive control for fungi, this is beside 50 µl of $AgNO_3$ solution in three different concentrations of 0.5, 1 and 2 mM. Plates were incubated @ 30°C for 24 and 48 h. for bacteria and fungi respectively. Thereafter, inhibition zone diameters in mm were recorded. The experiment was implemented in triplicates.

#### Antioxidant Activity of AgNPs

The capability of extract-based AgNPs to scavenge 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radicals was determined by mixing 100 µl of AgNPs solution in 1 ml of 0.1 mM DPPH methanolic solution. The mixture was agitated and incubated at 37°C for 30 min in dark, then the absorbance of samples was recorded at 515 nm. The DPPH scavenging activity was calculated using the following equation [41].

$$\text{DPPH scavenging activity (\%)} = (A^\circ - A_1)/A^\circ \text{ X } 100$$

where:

$A^\circ$ is the absorbance of the control reaction, $A_1$ is the absorbance of the reaction mixture.

## Cereal seed germination indices in response to cyanobacterial extracts/-based silver nanoparticles

Several germination attributes of four barley and wheat cultivars, two of either, were calculated to expound how far cyanobacterial extracts and/or their AgNPs might accelerate the seedling development. Petri dishes of 12 cm diameter with double layers of Whatman No. 1 filter papers were sufficiently moistened with either extracts or their AgNPs. Healthy seeds, similar in size and weight as possible, of barley cvs. Giza 123 and Giza 2000 as well as wheat cvs. Benisweif 7 and Misr 3 were the test plants. All cultivars are recommended for cultivation in marginal and newly reclaimed lands. Seeds were surface sterilized with 70% ethanol for 1 min, followed by immersion in 5% sodium hypochlorite for 10 min, then washed several times with sterilized distilled water. Seeds were submerged in the liquid tested materials for 20 min and distributed at the rate of 10 seeds $dish^{-1}$. Along a period extended for 10 days at ambient temperature, seed germination traits were monitored daily. Those included germination percentages at both the $1^{st}$ and the $10^{th}$

days, mean germination time (MGT), germination rate index (GRI), and germination velocity coefficient (GVC) according to the equations of Kader and Jutzi [42] and Kader [43]. The formulae used are:

MGT (days)=Σ (Ni Ti)/Σ Ni,

GRI (% $day^{-1}$)=Σ Ni/i,

and GVC=ΣNi/Σ (Ni Ti) ×100 (% $day^{-1}$);

where: N is the number of seeds germinated on day i while Ti is the number of days from seeding. Six isolates were used in this experiment.

### Statistical analysis

Statistical analysis of data was carried out using Assistate software [44,45]. based on one-way ANOVA and means were compared by least significant difference test ($p$, 0.05) according to procedures described by Snedecor and Cochran [46].

## Results

### Ecological, physico-chemical and cyanobacterial profiles of collected water samples

Biodiversity of cyanobacteria in different aquatic environments representing a number of preferable habitats of these microbiota was documented. Three major water sources including fresh Nile, marine and brackish waters were subjected for isolation of autochthonous members of cyanobacteria to guarantee a proper store of bioactive agents for biotechnological applications.

The physico-chemical characteristics of the experimented water samples are presented in (Table 2). The prevailing temperatures at sampling time relatively varied depending upon water type and source. In general, waters of the River Nile and Khadra Lake collected from Giza and El-Beheira (Wadi El-Natron) governorates respectively were the coldest (18°C), Red Sea sample of Suez (Ain Sokhna), on the other hand was the warmest recording a temperature of 26°C. Other water samples revealed temperatures falling in the range 19–24°C. The atmospheric pressure hardly fluctuated among the examined waters being in the range of 1010-1018 hPa. The pH estimates of waters indicated the neutrality to the alkaline side (7.7–8.9). As expected, considerable variations were scored in respect to electrical conductivity of samples. None of the examined 12 waters deemed non-saline, both River Nile samples of Giza and Damietta appeared saline with EC values of 6 and 7 $dSm^{-1}$, respectively. The only moderately saline water (EC, 12 $dSm^{-1}$) was that taken from the artificial pond located at the Faculty of Agriculture, Cairo University. The reminder 9 samples were categorized as highly saline with EC values ranging from 28 to 60 $dSm^{-1}$. Extraordinary EC record of 256 $dSm^{-1}$ was obtained for Khadra Lake water of Wadi El-Natron. Generally speaking, percentages of water salinity were more or less coping with measurements of electrical conductivity. No remarkable variations in water density were

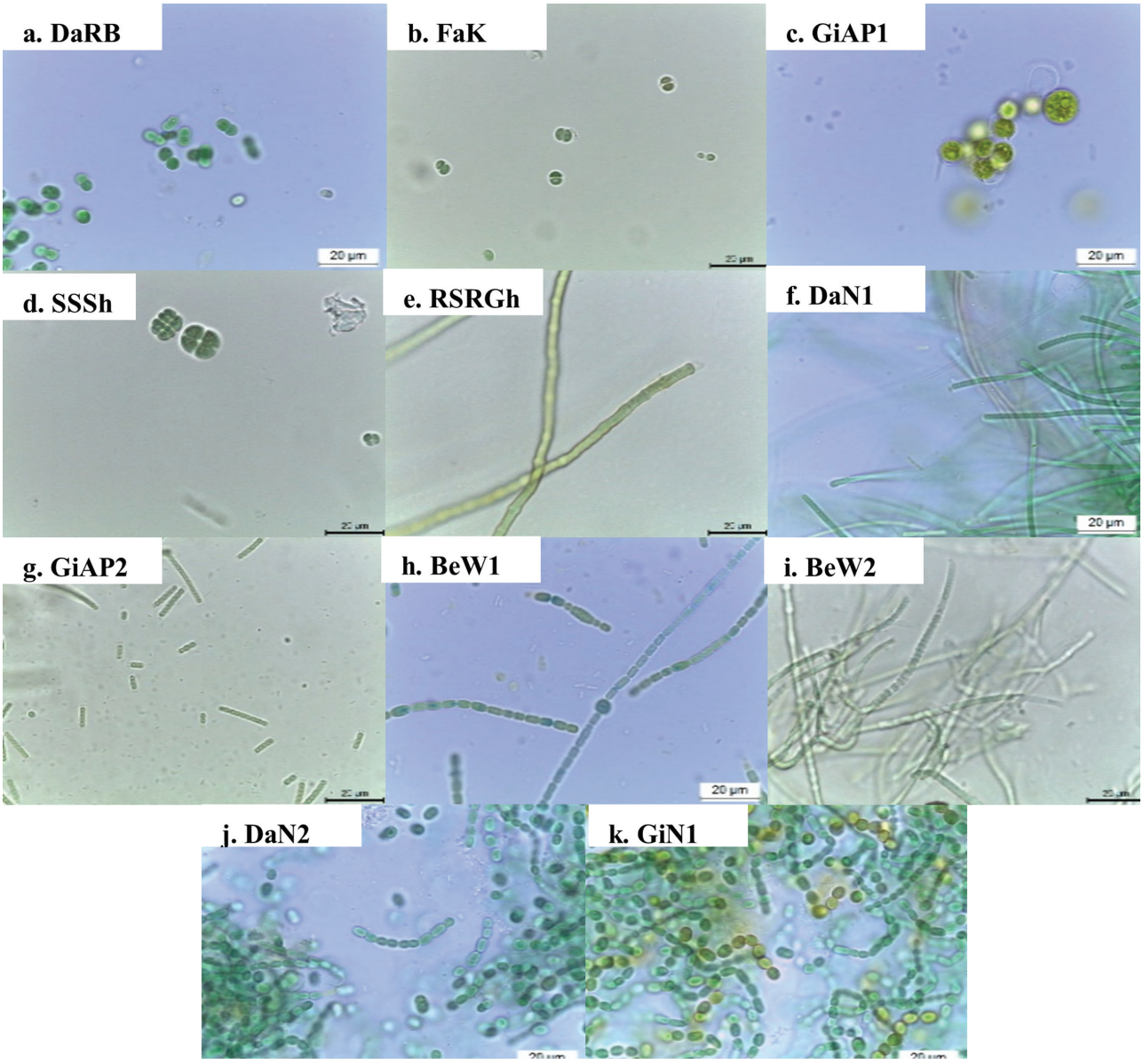
**Table 2 Ecological and physicochemical characteristics of collected water samples**

| Governorate | Site | Temperature (°C) | Atmospheric pressure (hPa) | pH | EC ($dSm^{-1}$) | Salinity (%) | Density ($gL^{-1}$) | Specific gravity | TDS ($mgL^{-1}$) | Dissolved $O_2$ ($mgL^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Giza | River Nile | 18 | 1017 | 8.4 | 6 | 3.8 | 1001.5 | 1.003 | 4800 | 10.2 |
| | Artificial pond (Fac. of Agric., Cairo Univ.) | 19 | 1017 | 8.9 | 12 | 7.9 | 1004.4 | 1.006 | 9600 | 3.0 |
| Damietta | River Nile | 20 | 1014 | 8.2 | 7 | 4.3 | 1001.5 | 1.003 | 5600 | 9.0 |
| | Mediterranean Sea | 24 | 1014 | 8.2 | 56 | 38 | 1025.9 | 1.029 | 44800 | 6.4 |
| | Ras El Bar estuary | 23 | 1014 | 8.1 | 28 | 18 | 1011.1 | 1.014 | 22400 | 6.0 |
| Alexandria | Mediterranean Sea (Sedi Krerr) | 21 | 1018 | 8 | 54 | 39.1 | 1027.6 | 1.03 | 43200 | 6.5 |
| Red Sea | Red Sea (Ras Ghareb) | 20 | 1016 | 7.9 | 55 | 40.9 | 1029.3 | 1.031 | 44000 | 5.4 |
| Suez | Red Sea (Ain Sokhna) | 26 | 1012 | 8.1 | 61 | 40.1 | 1026.9 | 1.03 | 48800 | 4.5 |
| South Sinai | Red Sea (Sharm El Sheikh) | 22 | 1010 | 8.1 | 57 | 40.6 | 1028.5 | 1.031 | 45600 | 4.3 |
| | Red Sea (Dahab) | 22 | 1012 | 8.1 | 55 | 39 | 1027.3 | 1.03 | 44000 | 5.0 |
| Fayoum | Karoun's Lake | 19 | 1015 | 7.9 | 60 | 46.2 | 1033.6 | 1.035 | 48000 | 6.5 |
| El-Beheira | Khadra Lake | 18 | 1018 | 7.7 | 256 | 308.4 | 1258.5 | 1.26 | 204800 | 3.8 |

observed among the collected samples, the density hardly exceeded 1000 g $L^{-1}$ in 11 samples except Khadra Lake water that recorded density of Ë 1200 g $L^{-1}$. Specific gravities of waters were almost akin among the tested samples (1.003–1.035), A relatively high specific gravity of 1.260 was recorded for Khadra Lake water. Total dissolved salts (TDS), as a key for water quality, were the lowest (4800–9600 mg $L^{-1}$) in Nile water samples irrespective of sampling sites. Khadra Lake marine sample scored an overestimated level of TDS of 20 4800 mg $L^{-1}$. Dissolved oxygen (DO), that speaks well on the quality of a water supply, conspicuously varied among the examined waters. As high as 9.0-10.2 mg $L^{-1}$ DO values were reported for Nile waters, the DO estimates of the other water samples ranged between 3.0 and 6.5 mg $L^{-1}$.

Light microscopic examinations of water samples showed relative occurrence of cyanobacteria that belonging to different species (data not shown). The relative occurrence of the microbiota seemed to be affected by several ecological factors such as temperature, lack of some trace elements required for growth of cyanobacteria besides the variable ratios among nitrogen, phosphorus and potassium as the key inputs necessary for the biological life sustainability. Fresh water samples showed higher relative occurrence than marine waters. Also, the results indicated that the presence of cyanobacteria was more pronounced during spring and early summer when the temperature ranged between 26 and 30°C while the absence or weak to moderate occurrence during winter season. Unexpectedly, the brackish water sample taken from Ras El Bar estuary

**Figure 1**




Light micrographs of purified cyanobacterial isolates, 1000x (description of the isolate morphologies are presented in Table 3).

appeared a favorite medium for living and persistence of the microorganisms.

#### Microscopic characterization and putative identification of cyanobacterial isolates

After enrichment and several successive transfers into the same cultivation media, cyanobacterial isolates were obtained by streak plate method or uni-filament isolation technique. The total 11 purified cyanobacterial isolates were morphologically identified based on light microscopic observations. The microscopic images of these isolates are presented in Fig. 1Fig. (1, a-k). The visual description and more details on the morphological features and proposed taxonomic positions of the isolates are presented in (Table 3).

## Characterization of biosynthesized cyanobacterial crude extract-based silver nanoparticles

#### Transmission electron microscopy (TEM)

The TEM micrographs of the formed cyanobacterial crude extract-based silver nanoparticles showed spherical shape of the majority with sizes varied from 7.3 to 28.0 nm (Fig. 2 and Table 4). The biosynthesizing conditions had variable influence on the produced materials. In this respect, higher temperatures relatively resulted in bigger nanoparticles, a finding that was cyanobacterial isolate-independent. Compared to darkness, light conditions seemed more favorable for bigger nanoparticle formation.

#### UV–Vis Spectrophotometry

The formation of AgNPs was detected by changing the particles from colorless to brown due to the excitation of SPR. Sharp peaks are given in the visible region from the UV-Vis spectra at 380 to 450 nm (Table 4) More details on the produced AgNPs are illustrated in (Fig. 3).

#### Fourier-transform infrared spectroscopy (FTIR)

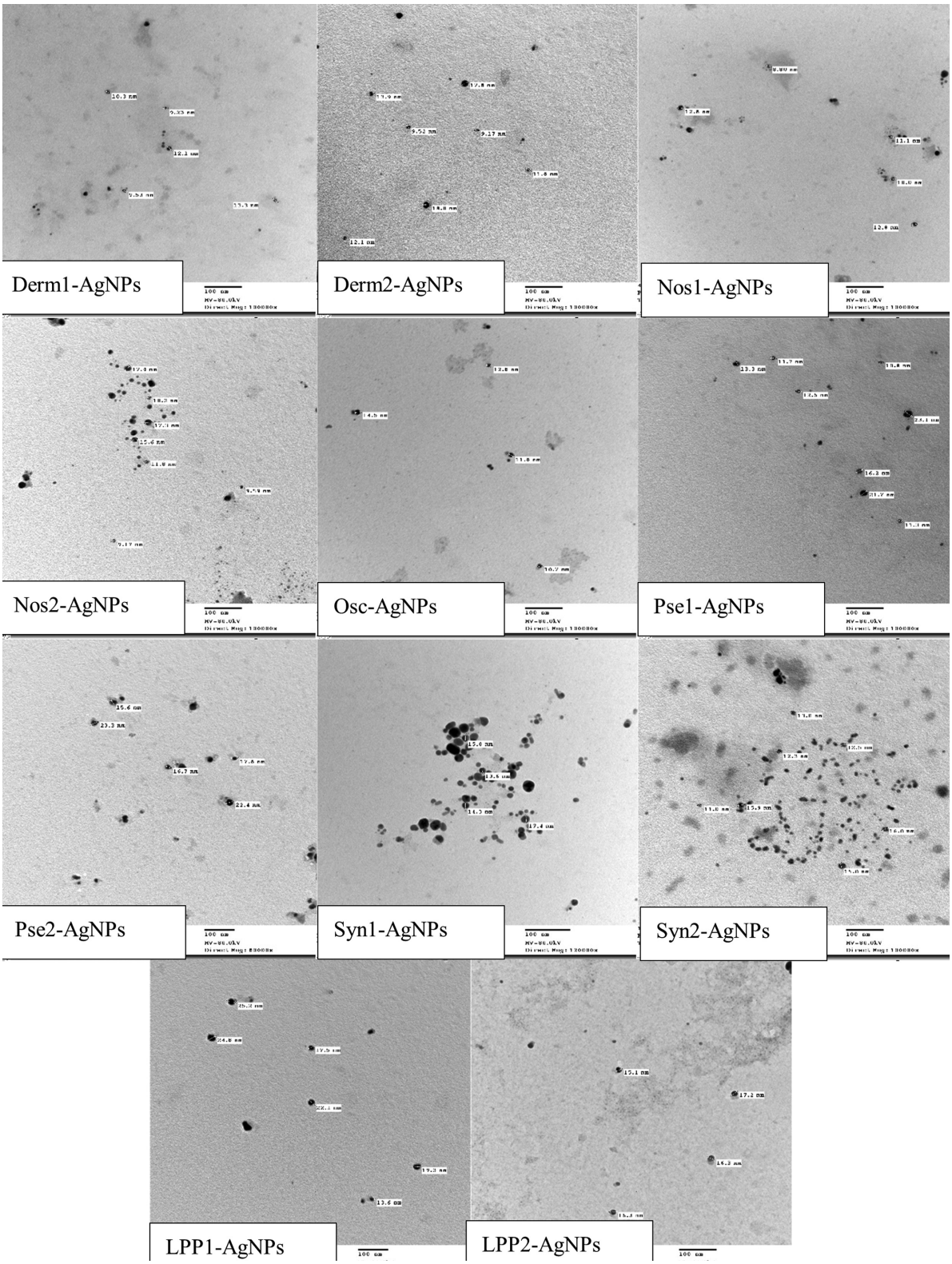
The FTIR spectra for cyanobacterial crude extracts showed the absorption bands at 1669, 1585, 1280, 994 and 768 $cm^{-1}$ for *Dermocarpella* sp.; 1671, 1582, 1426, 1273, 990, 818 and 517 $cm^{-1}$ for *Nostoc* sp.; 1658, 1583, 1281, 1175, 1004, 789 and 539 $cm^{-1}$ for *Oscillatoria* sp.; 1820, 1783, 1505, 1129 and 910 $cm^{-1}$ for *Pseudoanabaena* sp.; 1859, 1756, 1589, 1493 and 575 $cm^{-1}$ for *Synechocystis* sp. and 1849, 1811, 1540, 1132, 893 and 627 $cm^{-1}$ for LPP group A. While FTIR spectra of silver nanoparticles delivered from the crude extracts showed considerable shifting in

**Table 3 Morphological characteristics and taxonomic status of cyanobacterial members**

| Isolate code | Unicellular/ Filamentous | Cell shape | Cell Size (μm) | Heterocyst | Motility | Hormogonia | Akinetes | Sheath | Baeocytes | Gas Vacuole | Thylakoids | Subsection | Proposed genera |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DaRB | U | Spherical | 4.0 | - | - | - | - | - | - | - | + | I | *Synechocystis* sp. |
| FaK | U | Spherical | 3.5 | - | - | - | - | + | - | - | + | I | *Chroococcus* sp. |
| GiAP1 | U | Spherical | 5.5 | - | - | - | - | + | + | - | ND | II | *Dermocarpella* sp. |
| SSSh | U | Cubical cellular aggregates | 5.1*6.2 | - | - | - | - | + | + | - | ND | II | *Chroococcidiopsis* sp. |
| RSRGh | F | Disc-shaped | 3.7 | - | + | + | - | + | - | - | ND | III | *Oscillatoria* sp. |
| DaN1 | F | Disc-shaped | 2.0*1.0 | - | + | + | - | + | - | - | ND | III | LPP group A |
| GiAP2 | F | Isodiametric | 2.3*1.5 | - | + | + | - | - | - | + | ND | III | *Pseudanabaena* sp. |
| BeW1 | F | Cylindrical | 5.7*2.8 | + | + | + | + | - | - | - | ND | IV | *Anabaena* sp. |
| BeW2 | F | Disc-shaped | 2.7*1.9 | + | - | + | + | - | - | - | ND | IV | *Nodularia* sp. |
| DaN2 | F | Spherical, ovoid and cylindrical | 3.6*5.7 | + | + | + | - | - | - | - | ND | IV | *Nostoc* sp. |
| GiN1 | F | Spherical, ovoid and cylindrical | 3.6*5.7 | + | + | + | - | - | - | - | ND | IV | *Nostoc* sp. |

Isolate codes (refer to Table 1), U: unicellular, F: filamentous, ND: Not determined.

**Figure 2**




Electron micrographs of biosynthesized silver nano particles delivered from cyanobacterial crude extracts (more details are presented in Table 4).

wavenumber or changes in peak intensities as illustrated in (Fig. 4) The peak shifting suggests the responsible functional groups involved in the binding mechanism on the AgNPs.

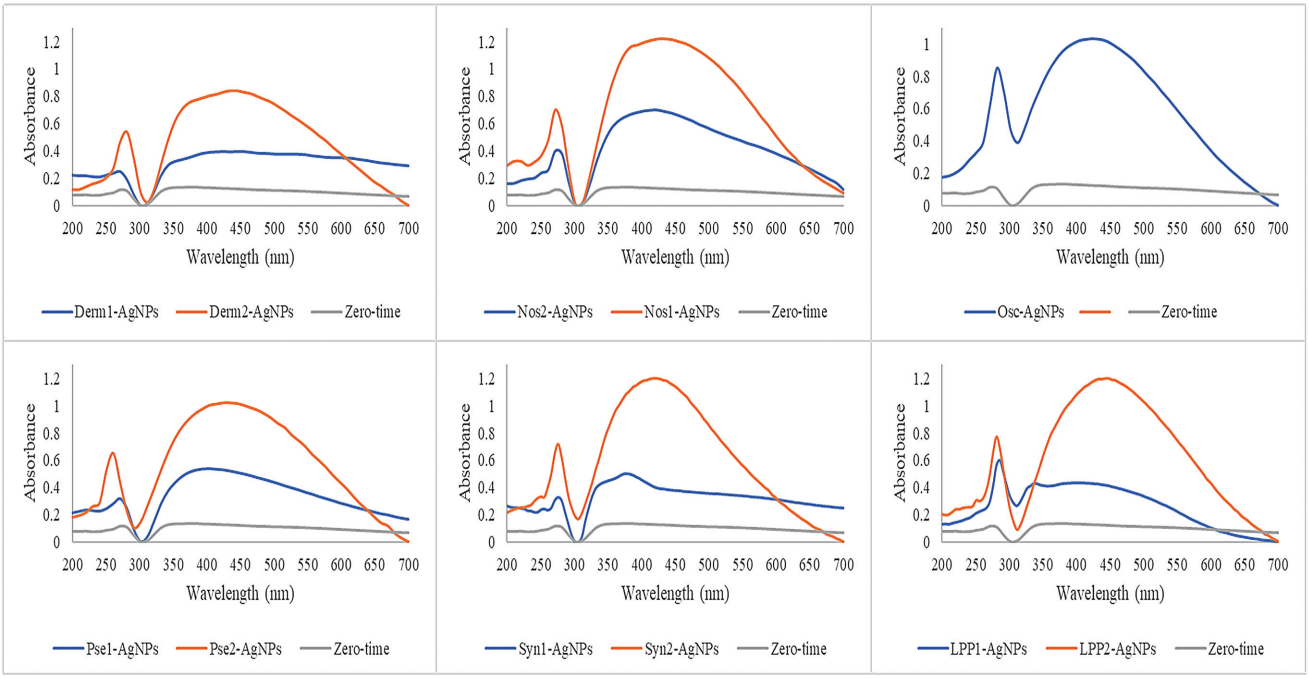
For Derm1-AgNPs, the peaks shifted to 1686, 1569, 1309, 1234 and 763 $cm^{-1}$ corresponding to C=O stretching, N-H bending, C-O stretching, C=C bending and C-H bending, while new peaks were

**Table 4 Biosynthesized silver nanoparticle characteristics**

| Culture extract | AgNPs Code | Biosynthesis cond. | | | Particle size (nm) | | SPR absorbance |
|---|---|---|---|---|---|---|---|
| | | L/D | Conc. $AgNO_3$ (mM) | Temp. °C | min. | max. | |
| *Dermocarpella* sp. | Derm1-AgNPs | L | 0.5 | 30 | 12.6 | 28.0 | 447 |
| | Derm2-AgNPs | L | 1.0 | 30 | 13.6 | 25.5 | 437 |
| *Nostoc* sp. | Nos1-AgNPs | L | 1.0 | 20 | 9.4 | 23.4 | 423 |
| | Nos2-AgNPs | L | 1.0 | 30 | 15.1 | 25.5 | 428 |
| *Oscillatoria* sp. | Osc-AgNPs | L | 1.0 | 40 | 13.6 | 25.7 | 424 |
| *Pseudoanabaena* sp. | Pse1-AgNPs | L | 1.0 | 20 | 10.8 | 23.1 | 425 |
| | Pse2-AgNPs | L | 1.0 | 30 | 7.8 | 18.7 | 406 |
| *Synechocystis* sp. | Syn1-AgNPs | L | 0.5 | 30 | 8.9 | 25.6 | 381 |
| | Syn2-AgNPs | L | 1.0 | 30 | 9.2 | 19.9 | 424 |
| LPP group A | LPP1-AgNPs | L | 0.5 | 20 | 7.3 | 25.3 | 411 |
| | LPP2-AgNPs | D | 1.0 | 30 | 8.8 | 19.9 | 443 |

L, Light; D, Dark; SPR, Surface plasmon resonance.

**Figure 3**




UV-Vis spectrophotometric graphs representing the cyanobacterial extract-based silver nanoparticles biosynthesized *via* various experimental conditions.

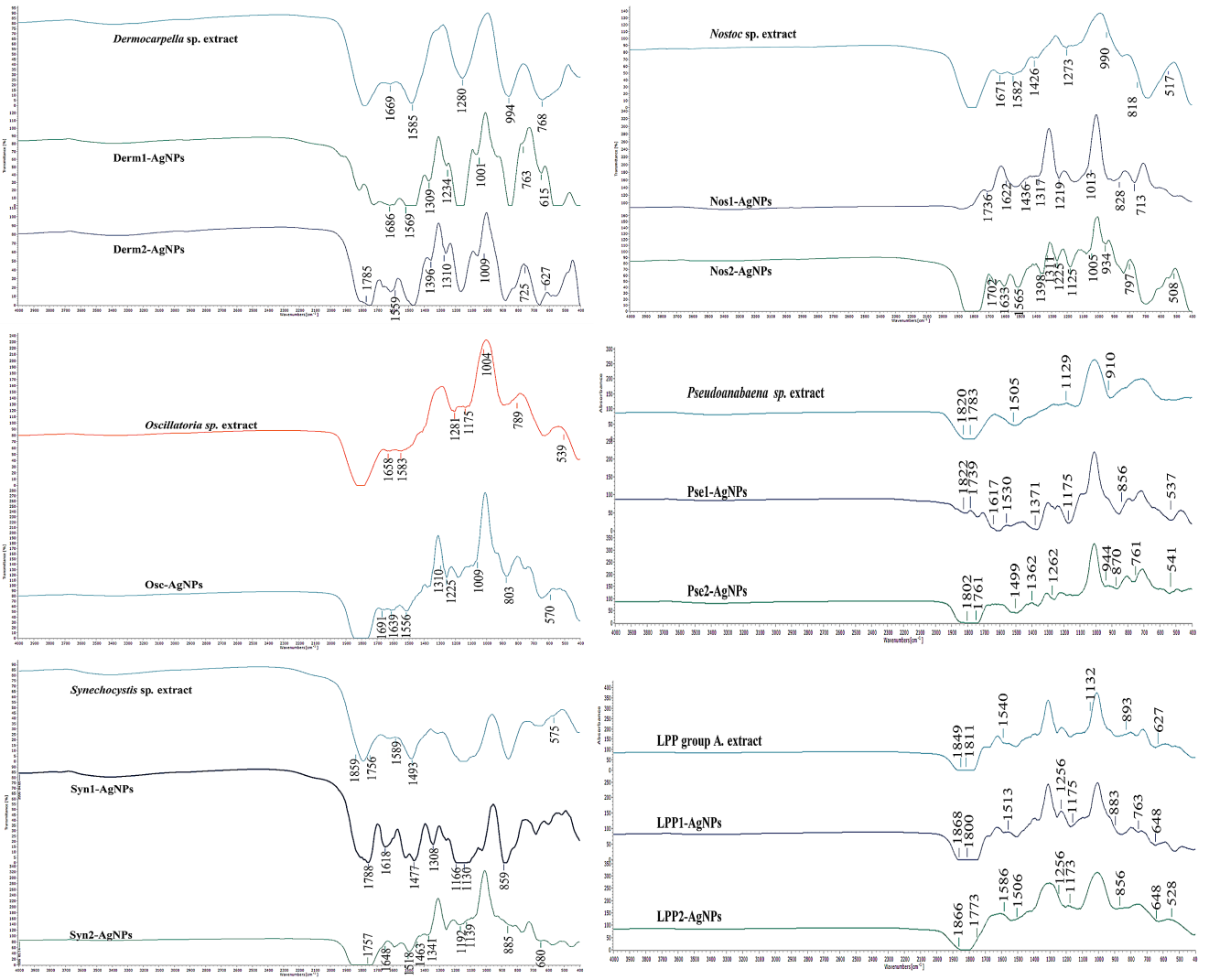
observed at 1001 and 615 $cm^{-1}$ corresponding to S=O stretching and C-Br stretching groups. In addition to shifting occurred to Derm2-AgNPs at 1702, 1565, 1225, 934 and 797 $cm^{-1}$, as well as 1310 and 627 $cm^{-1}$ observed as new peaks corresponding to S=O stretching group.

In respect to Nos1-AgNPs, the peaks shifted to 1736, 1622, 1436, 1219 and 828 $cm^{-1}$ corresponding to C=O stretching, C=C stretching, C-H bending, C-O stretching and, C=C bending. While a new peak was observed at 1317 $cm^{-1}$ corresponding to S=O stretching group. Besides, to shifting occurred for Nos2-AgNPs at 1702, 1565, 1225, 934 and 797 $cm^{-1}$; while 1633, 1398, 1311, 1125, 1005 and 508 $cm^{-1}$ were observed as new peaks.

In case of Osc-AgNPs, the peaks shifted to 1691, 1556, 1310, 1225, 1009, 803 and 570 $cm^{-1}$ corresponding to C=O stretching, N-H bending, C-O stretching, C-O stretching, S=O stretching, C-H bending, C-Br stretching and C=O stretching. With a new peak observed at 1639 $cm^{-1}$ corresponding to C=C stretching group.

For Pse1-AgNPs, the peaks shifted to 1822, 1739, 1530, 1175 and 856 $cm^{-1}$ corresponding to C=O stretching, C=O stretching, N-O stretching, C-O

**Figure 4**




FTIR spectra of cyanobacterial culture crude extracts compared to their corresponding biosynthesized silver nanoparticles (nos. 1 and 2 refers to biosynthesizing conditions indicated in Table 4.

stretching, C=C bending and C=O stretching; with new peaks observed at 1617, 1371 and 537 $cm^{-1}$ corresponding to C=C stretching and S=O stretching groups. Shifting, as well, occurred for Pse2-AgNPs 1802, 1761, 1262, 944 and 870 $cm^{-1}$; with 1499, 1362, 761 and 541 $cm^{-1}$ as new peaks corresponding to C-H bending, O-H bending and C-H bending groups.

Regarding Syn1-AgNPs, the peaks shifted to 1788, 1618, 1477, 1308 and 859 $cm^{-1}$ corresponding to C-H bending, C=O stretching, C=C stretching and C-H bending; in addition to a new peak observed at 1166 and 1130 $cm^{-1}$ corresponding to C-O stretching group. Shifting occurred, also, to Syn2-AgNPs 1757, 1648, 1518, 1463 and 680 $cm^{-1}$; with 1341, 1192, 1139 and 885 $cm^{-1}$ observed as new peaks corresponding to S=O stretching, C-O stretching, C-O stretching and C=C bending groups.

Akin to the previously mentioned materials, the LPP1-AgNPs peaks shifted to 1868, 1800, 1513, 1256, 883 and 648 $cm^{-1}$ corresponding to C-H bending, C=O stretching, N-O stretching, C-O stretching and C=C bending groups; with new peaks observed at 1175 and 763 $cm^{-1}$ corresponding to C-O stretching and C-H bending groups. In addition, shifting occurred for LPP2-AgNPs at1866, 1773, 1506, 1173, 856 and 648 $cm^{-1}$; while 1586, 1256 and 528 $cm^{-1}$ could be observed as new peaks corresponding to N-H bending and C-O stretching groups.

### Antimicrobial activity of cyanobacterial extracts/-based silver nanoparticles

When aqueous extracts of cyanobacterial fresh biomasses were assessed for antibiosis towards bacterial and fungal pathogens, none of the isolates showed apparent antibiosis. On the contrary, the extract-synthesized AgNPs successfully antagonized

**Table 5 Inhibition zone diameters, IZD (mm) of examined microbial strains attributed to cyanobacterial extracts-synthesized silver nanoparticles**

| AgNPs | *Bacillus cereus* | *Escherichia coli* O157: H7 | *Salmonella enterica* | *Staphylococcus aureus* | *Aspergillus niger* | *Candida albicans* |
|---|---|---|---|---|---|---|
| Derm1-AgNPs | 15 | 12 | 12 | 19 | 11 | 13 |
| Derm2-AgNPs | 15 | 12 | 13 | 20 | 11 | 13 |
| Nos1-AgNPs | 15 | 12 | 12 | 19 | 10 | 13 |
| Nos2-AgNPs | 15 | 11 | 12 | 19 | 11 | 13 |
| Osc-AgNPs | 16 | 12 | 11 | 19 | 11 | 13 |
| Pse1-AgNPs | 16 | 13 | 12 | 19 | 10 | 13 |
| Pse2-AgNPs | 15 | 12 | 11 | 20 | 10 | 13 |
| Syn1-AgNPs | 14 | 12 | 11 | 16 | 9 | 12 |
| Syn2-AgNPs | 15 | 12 | 13 | 19 | 11 | 13 |
| LPP1-AgNPs | 15 | 12 | 9 | 16 | 13 | 12 |
| LPP2-AgNPs | 15 | 12 | 10 | 19 | 10 | 13 |

LSD (0.05), 0.65; CV, 6.31%.

all the tested microbial pathogens, in a way or another. Irrespective of pathogenic microbiota, the cyanobacterium *Dermocarpella* sp. seemed the superior antagonist resulting in an average inhibition zone diameter (IZD) of *ca*. 14 mm (Table 5) *Pseudoanabaena* sp. ranked thereafter with IZD of 13.8 mm. The antimicrobial activities of the other cyanobacterial members were falling in the range 12.2–13.7 mm. In a number of cases, the nanoparticle biosynthesizing conditions of silver nitrate concentration, temperature and illumination had no significant influence on the cyanobacterial antibiosis against tested pathogens. Apart from cyanobacterial candidates, *Staphylococcus aureus* was the most susceptible pathogen recording an average IZD of 18.6 mm, this was followed by *Bacillus cereus* (IZD, 15.2 mm). *Aspergillus niger* strongly withstood the antagonistic impact of examined cyanobacteria, recording the least IZDs of 9–13 mm.

**Table 6 DPPH radical scavenging activity (%) of examined cyanobacterial extract-synthesized silver nanoparticles**

| | DPPH (%) | | |
|---|---|---|---|
| AgNPs | 10 | 15 | 20 |
| Derm1-AgNPs | 13.9 | 18.4 | 30.0 |
| Derm2-AgNPs | 62.1 | 85.0 | 96.0 |
| Nos1-AgNPs | 25.6 | 32.1 | 56.5 |
| Nos2-AgNPs | 23.6 | 32.1 | 50.0 |
| Osc-AgNPs | 43.1 | 56.0 | 80.0 |
| Pse1-AgNPs | 20.0 | 29.2 | 35.7 |
| Pse2-AgNPs | 39.3 | 50.0 | 70.2 |
| Syn1-AgNPs | 22.0 | 67.0 | 85.0 |
| Syn2-AgNPs | 58.8 | 70.2 | 88.0 |
| LPP1-AgNPs | 60.0 | 80.0 | 95.0 |
| LPP2-AgNPs | 55.0 | 77.0 | 93.0 |

LSD (0.05) for DPPH %, 20.13; CV, 16.40%.

### Antioxidant efficacy of cyanobacterial extract-based AgNPs

The antioxidant activity of 2,2-diphenyl-picrylhydrazyl (DPPH) in the concentrations of 10, 15 and 20% exhibited the efficient free scavenging of radicals (Table 6) The scavenging potential proportionally increased with increasing the DPPH concentration. Nanoparticles synthesized from *Dermocarpella* sp. extract with 1.0 mM $AgNO_3$ in light showed the highest scavenging activities of 62.1–96.0%. The LPP group of cyanobacteria, as well, deemed active in DPPH radical scavenging with activity percentages of 55.0–95.0. Compared to other isolates, *Nostoc* sp. appeared the inferior with antioxidant potentials of 23.6–56.5%. In general, variations in AgNPs biosynthesizing conditions had no remarkable influence on the antioxidant activities of the tested nanoparticles.

### Cereal seed germination as affected by cyanobacterial extracts/-based silver nanoparticles

Ten-day old barley and wheat seedlings were assessed for various germination attributes. As shown in (Table 7) seeds of both barley cultivars successfully germinated on the expense of all cyanobacterial culture extracts, an effect that relatively was isolate- or cultivar-dependent. The germination rate index (GRI) slightly varied among the microbiota, being the highest (20.0%) for LPP group A with cv. Giza 123 and 20.6% for *Oscillatoria* sp. with cv. Giza 2000. The germination velocity coefficient (GVC) hardly fluctuated among the applied treatments and ranged between 53.6 and 58.8% being somewhat higher than that calculated for distilled water-soaked seeds.

**Table 7 Germination traits of barley seeds soaked in the various cyanobacterial extracts**

| Cyanobacteria | Germination (%) | GRI (%) | GVC (%) | MGT (days) |
|---|---|---|---|---|
| **cv. Giza 123** | | | | |
| Distilled water | 100 | 18.0 | 55.3 | 3.3 |
| *Anabaena* sp. | 98 | 18.3 | 54.9 | 2.9 |
| *Chroococcidiopsis* sp. | 100 | 18.8 | 56.6 | 2.9 |
| *Chroococcus* sp. | 100 | 18.3 | 55.9 | 2.8 |
| *Dermocarpella* sp. | 100 | 18.9 | 54.2 | 3.0 |
| *Nostoc* sp. | 96 | 18.1 | 53.6 | 3.3 |
| *Oscillatoria* sp. | 100 | 19.9 | 56.1 | 2.8 |
| *Pseudanabaena* sp. | 97 | 18.9 | 56.9 | 2.6 |
| *Synechocystis* sp. | 100 | 18.2 | 54.9 | 3.4 |
| LPP group A | 97 | 20.0 | 58.8 | 3.0 |
| **cv. Giza 2000** | | | | |
| Distilled water | 97 | 18.8 | 54.1 | 3.3 |
| *Anabaena* sp. | 100 | 19.9 | 55.8 | 3 |
| *Chroococcidiopsis* sp. | 96 | 19.8 | 56.9 | 2.7 |
| *Chroococcus* sp. | 98 | 18.9 | 56.5 | 2.8 |
| *Dermocarpella* sp. | 100 | 18.7 | 56.8 | 3.0 |
| *Nostoc* sp. | 100 | 18.9 | 56.9 | 4.0 |
| *Oscillatoria* sp. | 96 | 20.6 | 59.9 | 2.5 |
| *Pseudanabaena* sp. | 98 | 19.6 | 56.6 | 2.8 |
| *Synechocystis* sp. | 100 | 19.8 | 55.9 | 2.9 |
| LPP group A | 96 | 18.8 | 58.7 | 2.9 |
| **LSD (0.05)** | | 0.65 | 1.62 | 0.96 |
| **CV (%)** | | 3.06 | 2.05 | 7.59 |

LPP group A (*Lyngbya, Plectonema* and *Phormidium*); GRI, germination rate index; GVC, germination velocity coefficient; MGT, mean germination time.

Variations among isolates and extracts were more remarkable in respect to mean generation times (MGT). While an average of 3.3 days was required for seed germination on distilled water of both barley cultivars, shorter times (< 3.0 days) were sufficient for germination of extract-immersed seeds. Generally, germination patterns of cv. Giza 2000 seeds were little bit better than the correspondings of the other cultivar. The average estimates of GRI, GVC and MGT were 19.4%, 56.8% and 3.0 days for the former and 18.7%, 55.7% and 3.0 days for the latter.

When barley seeds were allowed to germinate in presence of cyanobacteria extract-synthesized AgNPs, the situation slightly differed (Table 8). No obvious changes were recorded regarding the total germination percentages. Compared to germination on the extracts as such, both GRI and GVC increased being, in average, 19.3 and 56.3% for cv. Giza 123 as well as 19.6 and 56.9% for cv. Giza 2000 representing 3.7, 2.0, 1.6 and 1.1% increases, respectively. In addition, time required for seeds to germinate reduced from 3.0 to 2.7 days for cv. Giza 123, while cv. Giza 2000 showed slight reduction of mean germination time from 3.0 to 2.9 days. No obvious variations were monitored among the tested genera of cyanobacteria. Irrespective of isolate, nanoparticle synthesizing conditions ($AgNO_3$ concentration, temperature and illumination) and plant cultivar, the germination traits assessed were, generally, higher when the cereal seeds were treated by the extract-based AgNPs. This is expressed in 1.8 and 0.6 increase percentages in GRI and GVC respectively. As well, mean germination time reduced by 7.1%.

(Tables 9 and 10) present the results of germination profiles of wheat cvs. Benisweif 7 and Misr 3 seedlings when developed in presence of cyanobacterial extracts and their synthesized AgNPs. Apart from the tested isolates, seedlings delivered on extracts, in absence of the nanometal, recorded average GRI and GVC percentages of 19.0 and 58.7 for cv. Benisweif 7 against 19.3 and 61.3 for cv. Misr 3. Time needed for seed germination for both cultivars was similar (3.2 days, in average). The allowance to germinate in presence of the extract-based silver nanoparticles resulted in no apparent impact on germination attributes of the cereal plant except germination velocity coefficient which increased by only 0.9% for cv. Benisweif 7. As reported with barley cultivars, neither cyanobacterial genus nor AgNPs-synthesizing condition exhibited conspicuous variations in the measured germination traits.

## Discussion

Antibiotic resistance occupies a very special situation among the topics of the global agenda due to its reflection on public health, sustainable development, trade and global economy. Here, the necessity is raised to search for proper resources of the biosphere in the efficient and sustainable framework. The last few decades witnessed the growing of nanotechnology in the various scientific fields and exhibited beneficial roles for both humans and environment. Principally, nanoparticles of 1–100 nm in size are mainly classified in three distinct categories; a) organic materials that include dendrimers, liposomes, fullerenes, micelles, carbon nanotubes, carbon fibers, and lampblack graphene; b) inorganic nanoparticles that encompass noble metal, magnetic and semi-conductor nanoparticles, and c) composite nano-materials represented by combinations of carbon-, metal- and organic-based nanoparticles [47]. Among the well-known nanoparticles, those of silver could be synthesized adopting either physical, chemical or biological strategies. Physical techniques require high energy inputs by pressure and high temperature, while chemical procedures use different reducing and/or oxidative reagents *via* the sol-gel processes, spray pyrolysis, sputtering, chemical etching and atomic or molecular condensation [48].

Recently, great efforts have been done to adopt more safe and low-cost methods for silver-based nanoparticles production. Indeed, the biogenic synthesis of AgNPs is universally in use as friendly-environmental as well as cost-effective alternative strategy to the physical and chemical techniques. This study is among the on-going research attempts to synthesis AgNPs *via* a biological process, known as bio-nanofabrication, using the extracts of a number of cyanobacterial isolates secured from three different aquatic environments of Egypt. Out of eleven cyanobacterial extracts examined for silver-based nanoparticles formation, only six successfully delivered the nano-materials. Several analytical strategies were adopted in the present study to characterize the synthesized AgNPs. Ultraviolet-visible spectroscopy (UV-Vis spectroscopy) was used to assess the color change of the solution, where the bio-reduction of Ag ions to nano-Ag is done at a different wavelength range of 300-700 nm with a standard peak value in the range of 415–417 nm that indicates the synthesis of the metallic nanoparticles [49]. Other techniques are transmission electron microscopy (TEM), X-ray diffraction, and Fourier transform infrared spectroscopy (FTIR). Size, shape, mono-dispersity, aggregation nature, *etc*., are detected by TEM. The X-ray diffraction is a proper method usually applied to analyze the geometry of any unknown object, where the crystalline nature of the examined sample could be recognized by their diffraction patterns according to Bragg low [50]. The presence of some functional groups of carbonyl/amine/hydroxyl, that are involved in the reduction and capping of the nanoparticles during their synthesis could be detected by the FTIR analysis. Based on the implemented analyses in the present study, the synthesized AgNPs are characterized by spherical shape of different sizes. The crude extracts possessed a number of secondary metabolites that facilitate the formation of nano-materials.

While none of the tested extracts, as such, showed antimicrobial activities towards the examined bacterial and fungal pathogens (*Bacillus cereus*, *Escherichia coli* O157:H7, *Salmonella enterica, Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*), the microbiota extract-based AgNPs exhibited variable antibiosis patterns, a phenomenon that was microorganism-dependent. The cyanobacterium *Dermocarpella* sp. was the superior antagonist resulting in an average inhibition zone diameter (IZD) of *ca.* 14 mm, other cyanobacterial candidates showed IZD areas of 12.2–13.7 mm. In this context, several studies extensively used Ag-based nano-materials as antimicrobial agents to overcome the disadvantages of physico-chemical methods and antibiotic resistance. It is proved that AgNPs directly interact and penetrate the microbial cell wall causing sever disturbance in cell functioning resulting cell death [8,51]. These effects are depending upon AgNP shape, size, concentration, surface charge and colloidal state [52]. Sondi and Salopek-Sondi [53] found that silver nanoparticles did attach to the cell wall of *Escherichia coli* forming holes on the cell membrane that ultimately resulted in the death of the bacterium. The authors added that the size of the used material greatly affected their antibiosis potential, the smaller the particles the most active ones. Raffi *et al* [54] mentioned that AgNPs possess both bactericidal and bacteriostatic activities towards biofilm-producing microbiota. The AgNPs concentration of 60 $\mu g\,ml^{-1}$ seemed more effective against *Escherichia coli* leading to death of the bacterial cells due to its bactericidal effect. Tehri *et al* [55] reported that, the antimicrobial activities of silver nanoparticles are resulted from their: a) adhesion to the cell membrane of the organism that causing an alteration in its permeability, release of the internal constituents and impairment of transport

**Table 8 Cyanobacterial extract-AgNPs synthesized under different temperatures and illumination and their effects on seed germination of barley cultivars**

| AgNPs | Germination (%) | GRI (%) | GVC (%) | MGT (days) |
|---|---|---|---|---|
| **cv. Giza 123** | | | | |
| Distilled water | 100 | 18.9 | 55.3 | 3.3 |
| Derm1-AgNPs | 100 | 19.3 | 56.9 | 2.7 |
| Derm2-AgNPs | 100 | 19.6 | 55.8 | 2.9 |
| Nos1-AgNPs | 100 | 19.2 | 57.2 | 3.0 |
| Nos2-AgNPs | 99 | 19.6 | 58.4 | 2.6 |
| Osc-AgNPs | 100 | 19.4 | 55.4 | 2.1 |
| Pse1-AgNPs | 100 | 18.9 | 57.1 | 2.4 |
| Pse2-AgNPs | 100 | 20.0 | 56.2 | 3.0 |
| Syn1-AgNPs | 100 | 19.4 | 54.9 | 2.5 |
| Syn2-AgNPs | 100 | 19.9 | 54.8 | 2.6 |
| LPP1-AgNPs | 100 | 18.1 | 56.9 | 2.8 |
| LPP2-AgNPs | 100 | 18.9 | 56.7 | 2.8 |
| **cv. Giza 2000** | | | | |
| Distilled water | 97 | 18.8 | 54.1 | 3.3 |
| Derm1-AgNPs | 100 | 18.7 | 59.1 | 2.9 |
| Derm2-AgNPs | 100 | 19.2 | 55.9 | 2.9 |
| Nos1-AgNPs | 100 | 19.8 | 58.0 | 2.9 |
| Nos2-AgNPs | 100 | 20.0 | 55.3 | 2.6 |
| Osc-AgNPs | 100 | 19.9 | 58.9 | 3.0 |
| Pse1-AgNPs | 100 | 19.9 | 58.7 | 2.8 |
| Pse2-AgNPs | 100 | 19.8 | 59.2 | 2.2 |
| Syn1-AgNPs | 100 | 19.8 | 54.9 | 2.9 |
| Syn2-AgNPs | 100 | 20.8 | 55.0 | 3.4 |
| LPP1-AgNPs | 96 | 19.9 | 55.5 | 2.8 |
| LPP2-AgNPs | 100 | 18.9 | 58.7 | 2.9 |
| LSD (0.05) | 0.67 | 4.10 | 0.37 | |
| CV (%) | | 2.45 | 2.42 | 11.2 |

GRI, germination rate index; GVC, germination velocity coefficient; MGT, mean germination time.

**Table 9 Germination indices of wheat seeds treated with the various cyanobacterial extracts**

| Cyanobacteria | Germination (%) | GRI (%) | GVC (%) | MGT (days) |
|---|---|---|---|---|
| **cv. Benisweif 7** | | | | |
| Distilled water | 100 | 18.4 | 58.2 | 4 |
| *Anabaena* sp. | 100 | 19.4 | 59.2 | 3.4 |
| *Chroococcidiopsis* sp. | 98 | 19.8 | 59.9 | 3.3 |
| *Chroococcus* sp. | 100 | 18.9 | 59.2 | 3.6 |
| *Dermocarpella* sp. | 100 | 19.2 | 56.4 | 2.9 |
| *Nostoc* sp. | 96 | 17.9 | 59 | 3.3 |
| *Oscillatoria* sp. | 100 | 19.3 | 59.1 | 2.9 |
| *Pseudoanabaena* sp. | 100 | 19.8 | 60.2 | 3 |
| *Synechocystis* sp. | 98 | 17.9 | 56.4 | 2.8 |
| LPP group A | 100 | 18.9 | 59.3 | 3.1 |
| **cv. Misr 3** | | | | |
| Distilled water | 100 | 19 | 60.4 | 4 |
| *Anabaena* sp. | 100 | 19.9 | 61.9 | 3.6 |
| *Chroococcidiopsis* sp. | 100 | 18.9 | 61.9 | 3.1 |
| *Chroococcus* sp. | 100 | 18.8 | 62.1 | 2.9 |
| *Dermocarpella* sp. | 100 | 20 | 60.2 | 2.9 |
| *Nostoc* sp. | 100 | 19.4 | 61.1 | 3.1 |
| *Oscillatoria* sp. | 100 | 18.9 | 61.6 | 3.6 |
| *Pseudoanabaena* sp. | 100 | 18.9 | 59.9 | 2.8 |
| *Synechocystis* sp. | 100 | 19.5 | 62.7 | 2.9 |
| LPP group A | 100 | 20 | 61.1 | 3.1 |
| LSD (0.05) | | 0.79 | 0.87 | 1.06 |
| CV (%) | | 3.40 | 1.97 | 7.83 |

LPP group A (Lyngbya, Plectonema and Phormidium); GRI, germination rate index; GVC, germination velocity coefficient; MGT, mean germination time.

**Table 10 Cyanobacterial extract-based AgNPs synthesized under various production conditions and their impacts on wheat germination attributes**

| AgNPs | Germination (%) | GRI (%) | GVC (%) | MGT (days) |
|---|---|---|---|---|
| **cv. Benisweif 7** | | | | |
| Distilled water | 100 | 18.4 | 58.2 | 3.4 |
| Derm1-AgNPs | 100 | 18 | 59.4 | 3 |
| Derm2-AgNPs | 100 | 19 | 59 | 2.8 |
| Nos1-AgNPs | 98 | 18.7 | 60.1 | 3.1 |
| Nos2-AgNPs | 100 | 19.9 | 60.3 | 2.9 |
| Osc-AgNPs | 100 | 19.6 | 59.4 | 2.8 |
| Pse1-AgNPs | 100 | 18.4 | 59.4 | 3.1 |
| Pse2-AgNPs | 100 | 18.9 | 58.8 | 2.8 |
| Syn1-AgNPs | 98 | 19.9 | 57.9 | 2.8 |
| Syn2-AgNPs | 100 | 19.8 | 60 | 3 |
| LPP1-AgNPs | 100 | 18.7 | 59.7 | 2.7 |
| LPP2-AgNPs | 100 | 17.9 | 58.8 | 2.7 |
| **cv. Misr 3** | | | | |
| Distilled water | 100 | 19 | 60.4 | 3.6 |
| Derm1-AgNPs | 100 | 19.6 | 60.9 | 3.5 |
| Derm2-AgNPs | 100 | 19.8 | 60.5 | 3 |
| Nos1-AgNPs | 97 | 18.8 | 62.1 | 2.8 |
| Nos2-AgNPs | 96 | 18.9 | 62.2 | 2.9 |
| Osc-AgNPs | 100 | 20 | 61.2 | 3.1 |
| Pse1-AgNPs | 99 | 19.3 | 59.9 | 3.4 |
| Pse2-AgNPs | 100 | 18.6 | 60.9 | 2.6 |
| Syn1-AgNPs | 100 | 19.8 | 61.4 | 3 |
| Syn2-AgNPs | 100 | 19.9 | 60.9 | 2.7 |
| LPP1-AgNPs | 98 | 18.9 | 62.0 | 3 |
| LPP2-AgNPs | 100 | 18.8 | 60.8 | 2.8 |
| **LSD (0.05)** | 0.74 | 0.50 | 0.69 | |
| **CV (%)** | | 2.50 | 0.88 | 6.11 |

LPP group A (*Lyngbya, Plectonema* and *Phormidium*); L/D, light/dark; GRI, germination rate index; GVC, germination velocity coefficient; MGT, mean germination time.

system, b) intracellular invasion resulting in destabilization and denaturation of cell protein beside interaction with DNA, c) generation of free radicals and reactive $O_2$ causing undesirable oxidation of both cell proteins and lipids, disturbing the mitochondria function, and d) genotoxicity encompassing the damage of DNA bases, strands break and mutation as well as suppression of replication and transcription.

The antioxidant activity of 2,2-diphenyl-picrylhydrazyl (DPPH) in the concentrations of 10, 15 and 20% exhibited the efficient free scavenging of radicals. Nanoparticles synthesized from *Dermocarpella* sp. extract with 1.0 mM $AgNO_3$ in light showed the highest scavenging activities of 62.1–96.0%. In this respect, Koleva *et al* [56]. mentioned that several cyanobacterial strains and medicinal plant species have free radical scavenging molecules including phenolic compounds, vitamins and terpenoids besides some other indigenous metabolites, those materials were found to have exceptional antioxidant activity.

Even if silver-based nanoparticles are having tremendous advantages and benefits in various application fields of medicines, pharmaceuticals, electronics, cosmetics and others; but they might be hazardous depending on the synthesis approach [57]. Upon continuous use, they form aggregates that might possibly toxic to a given environment, therefore, their stabilization is of special importance. Actually, stabilization of AgNPs could be employed using some capping materials and/or surface modifiers. Such materials must carefully be chosen as they perhaps result in some changes in the physico-chemical properties of AgNPs. The selected agents should principally be non-toxic, bio-soluble, bio-degradable, well dispersed, and bio-compatible [57]. As reported by Hamouda *et al*. [58], the antimicrobial potential of silver nanoparticles capped with Sodium Dodecyl Sulphate (SDS) exceeded the corresponding without SDS. They came to the conclusion that the use of biological methods simultaneously with external capping agents is much better in AgNPs antibiosis experimentation. Several *in vitro* investigations demonstrated the AgNPs toxicity in rat hepatocytes,

neuronal cells, murine stem cells as well as human lung epithelial cells [59,60]. *In vivo* assays, as well, toxicity studies using a rat ear revealed that exposure to silver nano-materials led to obvious mitochondrial dysfunction and consequent temporary and/or permanent hearing loss, an effect that was inoculation dose-dependent. Even the very low levels of the nanoparticles were absorbed by the retinal cells leading to a great structural disruption, this was attributed to the increased number of cells which underwent oxidative stress [61]. Wu *et al*. [62] reported that variations in surface charge as a result of surface functionalization of silver nanoparticles might affect cellular uptake, translocation to different tissues and cytotoxicity. The authors added that, the magnitude of surface charge, that determined by zeta potential, possibly affect the quantity of nano-materials and their mechanism of uptake inside cells. As reported by Ivask *et al*. [63] and Durán *et al*. [64], the cytotoxicity and genotoxicity of silver-based nanoparticles are generally affected by a number of physico-chemical features, *e.g.*, dispersion rate, level of application, size, surface charge and morphology beside surface functionalization. But until recently, the results of the implemented trials deem insufficient in respect to the accurate toxic impacts of such nano-materials in addition to their related mechanisms of toxicity.

Actually, small farms and private sectors, particularly in developed countries, require special and great support from the governments as well as the financial supporting agencies, as they looking for increasing farm products besides improving the efficient use of land water resources. Among the most considered ways to improve crop productivity, accelerating seed germination and seedling development occupies a non-tiny situation. Several metallic nano-materials have recently received a great attention due to their environmentally friendly applications in agriculture. Indeed, climate change and steady increasing population represent threatening global agricultural food security. Abiotic stresses in the environment including drought, extreme high and low temperatures in addition to salinity are significantly affecting seed germination, plant development and productivity. A number of metal-based nanoparticles (AgNPs, AuNPs, FeNPs, TiNPs, ZnNPs) have nowadays been used to modify/improve seed germination, plant growth, and stress tolerance of some crop plants [13]. In the present study, both cyanobacterial extracts and their silver-based nanoparticles were experimented for seed germination attributes of some barley and wheat cultivars. Results showed positive impacts of the used AgNPs on all the assessed germination traits, *i.e.* total germination (TG, %), germination rate index (GRI, %), germination velocity coefficient (GVC, %) and mean germination time (MGT, days). The supporting influence of extract-silver nanoparticles exceeded that of the extract as such. The promoting impacts of AgNPs on seed germination was previously reported in other investigations. Studies of Kale *et al* [15] scored increases in seed germination index, seed germination potential, mean germination time, seed vigor index, in addition to remarkable increases in seedling fresh and dry weights.

## Conclusion

In conclusion, silver-based nanoparticles possess unique properties that have great applications not only limited to medicine but for agriculture as well. This posits that nanotechnology is considered a new field of revolutionary science. Green synthesis largely involves the use of various plant species and microbes. Cyanobacterial-mediated synthesis could be achieved using the cell extract, but despite recording a number of advantages, the production of AgNPs is still relatively limited in use. This, actually, attributed to the lack of sufficient knowledge on the real mechanisms of synthesis.

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### Conflicts of interest

There are no conflicts of interest.

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