Algal Biomass Extract as Mediator for Copper Oxide Nanoparticle Synthesis: Applications in Control of Fungal, Bacterial Growth, and Photocatalytic Degradations of Dyes

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Recently, algae have attracted the attention of investigators as a renewable source of compounds that can contribute to nanoparticle creation. The use algae biomass to facilitate preparation of copper oxide nanoparticles (CuONPs), as well as their application, were the aims of the present study. High performance liquid chromatography analysis of algal biomass indicated the presence of daidzein (2550 µg/mL), followed by ellagic acid (596 µg/mL). Algal biomass extract was successful as a bio-reducing agent for CuONPs fabrication at different temperatures up to 50 °C. Transmission electron microscopy characterized the created CuONPs with average size 5 to 17 nm. The colony radius of *M. anisopliae*, *T. harzianum*, *C. lunata*, *F. oxysporium, A. flavus*, and *A. terreus* was 1.84 ± 0.08, 1.97 ± 0.03, 1.00 ± 0.08, 2.04 ± 0.03, 2.32 ± 0.06, and 2.42 ± 0.05 cm, respectively at 200 mg of CuONPs. CuONPs exhibited inhibition zones of 26, 23, 25, and 22 mm when tested against *B. subtilis, E.coli, K. pneumoniae,* and *S. aureus*, respectively. Methyl orange and methyl green dyes were degraded by CuONPs with percentages ranging from 9.5 to 63.7% and from 22.3 to 75.7% at 15 to 90 min, respectively. Therefore, the created CuONPs can be regarded as excellent candidates for controlling fungal/bacterial development and dyes degradation.

DOI: 10.15376/biores.18.4.7474-7489

Keywords: Algae; Nanoparticles; Fungi; Control; Photo-catalytic; Dyes; Degradation

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INTRODUCTION

The synthesis of eco-friendly nanoparticles (NPs) is an interesting area in nanobiotechnology. Biological processes to make NPs can be viewed as promising substitutes to chemical routes, as they have potential to avoid the generation of environmental impacting secondary pollutants. The green creation of NPs uses reductant agents from living organisms. Various biological creators of NPs, including algae, bacteria, fungi, and plants, have been documented in several studies (Abdelghany *et al.* 2018; Sharma and Kumar 2021; Al-Rajhi *et al.* 2022a; Abdelghany *et al.* 2023). These biological creators possess different molecules, such as polysaccharides, amino acids, alcoholic compounds, alkaloids, and vitamins responsible for stabilizing and reducing nanoparticles (Waris *et al.* 2021).

Algal biomass has been investigated as a prospective option for the environmentally friendly production of copper oxide nanoparticles (CuONPs). It is offered as a unique and simple process. The function of certain biomolecules and their contribution as capping and reluctant agents have not yet been fully investigated. The production of nanoparticles is made non-toxic and ecologically friendly using micro algae extracts as capping and reducing agents. When compared to other microorganisms, algae's rapid growth and high biomass productivity have the added benefit of making the process more affordable. Algae may flourish in both clean water and effluent, which further enhances its environmental friendliness. Algae are being used extensively in the creation of nanoparticles. They are referred to as "bionanofactories" because they produce nanoparticles using both living and dead biomass of algae. Algae have a high capacity for absorbing metals, making the biological process that uses them economical and environmentally acceptable (Bilal *et al.* 2018). Large amounts of the reducing agent, which transforms metal salts into their corresponding metal nanoparticles without producing any harmful byproducts, are present in algae. Secondary metabolites, including polysaccharides, proteins, tannins, and steroids, are present in the aqueous extract of algae as bioactive compounds (Jin *et al.* 2016).

Because of their larger surface area per weight or volume and numerous characteristics, metal NPs have gained attention in recent years. Their thermal, biological, chemical, electrical, dielectric, physical, magnetic, mechanical, and optical properties make them appealing tools for research work (Khan *et al.* 2017). In a variety of areas, including medicine, screening, drug administration, antisensory, tissue biotechnology, cosmetics, gene engineering applications, and several others, nanoparticles play the most significant role (Sharma and Sharma 2017). Among the various metal oxide nanoparticles, copper oxide has received particular attention, as copper-based compounds possess effective biocidal properties and thus can be used in the formulations of pesticide and other health-related applications.

The antifungal activity of compounds containing copper has been reported and applied for a long time. It is still being applied today, even with its associated environmental problems. Many researchers recommend substitution of copper with CuONPs. Several fungi, including *Fusarium culmorum*, *F. oxysporum*, *F. graminearum*, *F. solani*, *Aspergillus niger*, *A. flavus*, *Penicillium chrysogenum*, and *Alternaria alternata* are inhibited by CuONPs (Shende *et al.* 2015; Abdelghany *et al.* 2020). Fungicidal activity of CuONPs was recorded on the genetic levels of *Penicillium digitatum* and *F. solani* (Khamis *et al.* 2017). Ultrastructure changes of *F. incarnatum* were recorded, such as injury of hypha, conidiospores, cell membranes, and walls, as result of treatment by CuONPs (Al-Rajhi *et al.* 2022b).

Several environmental problems arise and increase each year as a result of the discharge of dyes into water bodies (Mehra *et al.* 2021). Therefore, the search for new or development of approaches for dye degradation is a challenge for investigations (Abdelghany *et al.* 2019; Ihsanullah *et al.* 2020; Nemiwal *et al.* 2021; Qanash *et al.* 2023). Numerous semiconductors or metal oxides are efficient photocatalysts under different sources of light, because of their small band gaps. For instance, a small band gap ranging from 2.1 to 2.71 eV is associated with CuO (Karthikeyan *et al.* 2020). CuO NPs display an appropriate response towards mechanical, optical, and photolytic applications (Pourmoslemi *et al.* 2020). Algae are recognized for accumulating heavy metals and also have a remarkable capacity to transform them into more pliable forms. Because of these enticing characteristics, algae have been anticipated as model organisms for producing different forms of nanomaterials, particularly metallic NPs (Fawcett *et al.* 2017).

Moreover, the environmental habitat of the collected biomass in the present study is rich with numerous minerals as well as the abundance of theses algae without any benefits. For these reasons, the algal biomass was used to create CuONPs with some applications including antifungal activity and dyes degradation.

EXPERIMENTAL

Source of Algal Biomass

Abundant green algae were developed in an agricultural drainage channel located at Monufia Governorate, Egypt (30° 62′8014″ N, 116° 31′ 070334″ E). This channel contains polluted water from different sources, including municipal, agricultural runoff, and industrial wastewater (Abdel Ghany *et al.* 2021). Fresh biomass (250 g) was collected from this channel and rinsed several times with distilled water. Then, the rinsed biomass was shade-dried, and 10 g of algal biomass were ground and immersed in 200 mL of distilled water, followed by simultaneously autoclaving and filtrating using filter paper (Whatman No. 1). Through centrifugations, the resulting supernatant from the filtered extract was utilized as the reducing agent for creating CuONPs.

Phenolic and flavonoid constituents of algal biomass

The washed and shade-dried algal biomass (50 g) was ground and extracted with 250 mL of methanol (20% w/v). The extract was subjected to high performance liquid chromatography (HPLC; Agilent 1260 Infinity HPLC Series, Agilent Technologies, Santa Clara, CA, USA) to detect the flavonoid and phenolic contents**.** The HPLC was fortified with a Quaternary pump and a Zorbax Eclipse amended with a column of C18 (100 mm × 4.6 mm i.d.). Twenty µL of the extract were injected in HPLC. Three gradient elutions were applied for the phenolic constituents separation at 30 °C including HPLC grade water 0.2% H_3PO_4 (*v*/*v*), methyl alcohol (B), and acetonitrile (C). The detector wavelength was applied at 284 nm. For flavonoid constituents' separation, the Knauer HPLC was fortified by a binary pump, the applied gradient elutions consisted of methanol and 0.5% of H_3PO_4 in water (50:50 %) with 0.7 mL/min of flow rate. Twenty µL of algal extract were injected. The detector wavelength was applied at 284 nm for flavonoids detection. The identification of constituents was depended on the existence of standard constituents.

Biogenic synthesis of copper oxide nanoparticles.

With vigorous stirring for 1 day at 100 °C, 10 mL of algal extract (5% g/v) was added dropwise to 100 mL of 1 mM aqueous copper acetate in an Erlenmeyer flask (250 mL capacity) for synthesis of CuONPs as a positive reaction mixture. Moreover, copper acetate aqueous solution without algal extract was used as negative control and kept at the same conditions of the positive reaction mixture. If the color in positive reaction mixture was changed after 5 h from bright blue to dark brown, it was a sign that CuONPs were forming, but in the negative control, the color was unaltered. Regular color changes and UV-visible spectrum measurements were made to track the process's development. The formed CuONPs were collected *via* centrifugation process for 15 min. The resulting CuONPs were then redispersed and cleaned by deionized H_2O to remove any debris and uncoordinated biomolecules. This separation and washing procedure was repeated three times to ensure CuONPs separation. The obtained pure CuONPs were oven-dried to complete the characterization process (Shehabeldine *et al.* 2023).

Characterization of created CuONPs

The creation of CuONPs by algal biomass was documented *via* a UV-visible spectrophotometer (Nicolet evolution 100, Cambridge, MA, USA) in the wavelength range (200 to 700 nm). Additionally, the reaction mixture at different temperatures (30, 40, and 50 ℃) was evaluated using an UV-visible spectrophotometer for synthesis of CuONPs. Shape and size of created CuONPs were investigated using a transmission electron microscope (TEM; JEOL JEM-2100, Tokyo, Japan). The created CuONPs were suspended in aqueous solution; and then a suspension drop was transported onto the TEM grids, and then dried before examination. An X-ray diffractometer X'Pert Pro (Philips, Eindhoven, Netherlands) was applied to evaluate the crystallinity of CuONPs created by algal biomass. The temperature range of 2θ was 4 to 70 °C. The radiation of Ni-filtered Cu Ka was utilized as a source of X-ray, with 40 kV as voltage and 30 mA as current.

Antifungal and antibacterial activity

Various phytopathogenic (*Curvularia lunata* and *Fusarium oxysporium*), mycotoxigenic (*Aspergillus flavus* and *Aspergillus terreus*), and bio-applicable (*Metarhizium anisopliae* and *Trichoderma harzianum*) fungi were used for testing. Petri dishes contained a solid culture medium without tested compounds (CuONPs, copper acetate, algal extract, and copper oxychloride (chemical fungicide)), and a medium fortified with different concentrations of each tested compound (50, 100, and 200 mg/L). Fungal mycelia (6 mm of fungal disc) were transferred to the center of the agar plate's surface, and then incubated at 30 °C for 7 days. Growth development of the tested fungi was estimated *via* measuring the colony radius compared to the control cultures (Abdelghany *et al.* 2015).

Nutrient agar plats were inoculated with tested bacteria including *Staphylococcus aureus* ATCC6538, *Bacillus subtilis* ATCC6633, *Escherichia coli* ATCC8739, and *Klebsiella pneumoniae* ATCC 8047 *via* a streaking method. Then, discs (6 mm) loaded with 100 µL of CuONPs, copper acetate, algal extract, and Gentamycin as standard antibiotic (20 µg/mL), were placed on the surface of inoculated agar with the tested bacteria. The plates were kept in refrigerator for 30 min to allow the diffusion of tested materials before bacterial growth, then incubated at 37 for 24 h, and then the appeared clear zones were measured (Humphries *et al.* 2018).

Photocatalytic test of CuONPs

According to Abdelghany and Al Abboud (2014) with some modification, the ability of CuONPs to degrade the methyl orange (MO) and methyl green (MG) dyes was estimated in the presence of visible light (500 km $h^{-1}m^{-2}$ as a mean solar flux). Each dye at concentration 10 mgL^{-1} was mixed with 10 mg of CuONPs. Then, for 30 min, the reaction mixture was agitated in the dark to authenticate the equilibrium of adsorption–desorption. A well-established spectrum of UV-vis absorption was seen in all experiments. Bands at 632 nm and 462 nm were scanned for MG and MO. The mixture reaction was then stirred under irradiation of sunlight, followed by withdrawing two mL of the reaction mixture each 15 min, up to 90 min, to detect the peak of absorption *via* UV-Vis at time "*t*". To calculate the dye degradation, the following equation was used:

$$\text{Degradation}(\%) = \left(\frac{\text{Absorbance at time, 0} - \text{Absorbance at time, t}}{\text{Absorbance at time, 0}}\right) \times 100$$

Statistical Assessment

One-way analysis of variance (ANOVA) in CoStat software (version 14, IBM Corp., Armonk, NY, USA) was applied to statistical studies, with significant differences detected *via* Tukey's test *post hoc* besides standard deviation (SD) approaches. The differences in results with the similar letters are not significant.

RESULTS AND DISCUSSION

The water surface was covered with green algal biomass for a long time (Fig. 1A). In each period these algal biomass were removed (Fig. 1B) or disappeared as suitable conditions in the water were unavailable. The appearance of these algae is considered a bio-indicator of water content changes. Some kinds of algae develop in complex habitats, and they can live in extreme conditions such as temperature, salinity, ultraviolet radiation, and nutrients; therefore, to continue, they must adapt to stress conditions *via* producing various secondary metabolites (Sirbu *et al.* 2020). The HPLC analysis of algal biomass indicated that daidzein represented the most identified compounds with a concentration of 2550 µg/mL, followed by ellagic acid (596 µg/mL). Moderate concentrations were associated with caffeic acid (262 µg/mL), gallic acid (233 µg/mL), and chlorogenic acid (205 µg/mL). Some compounds were detected in very low concentrations, such as ferulic acid (7.93 µg/mL), cinnamic acid (18.6 µg/mL), methyl gallate (18.9 µg/mL), and apigenin 22.6 µg/mL). According to the availability of standard phenolics and flavonoids, five compounds not detected in algal biomass included catechin, syringic acid, pyro catechol, rutin, coumaric acid, vanillin, and hesperetin. In contrast, three compounds at different retention times were identified; however, these compounds occurred with high area (%) (Table 1 and Fig. 2). As mentioned, algae are a unique natural biomass as a source of a number of compounds with several valuable properties. Brodowska (2017) identified many constituents, such as daidzein, kaempferol, naringenin, and apigenin, in green algae. Quercetin, kaempferol, and naringenin were also detected in many algal extracts (Gentscheva *et al.* 2022).

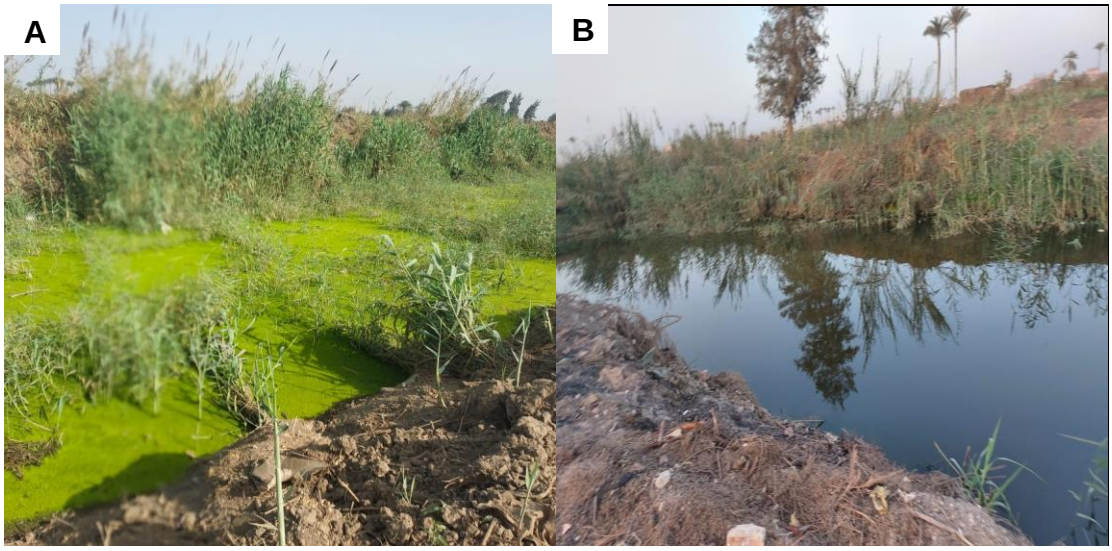

Fig. 1. Collection site of algal biomass in agricultural drainage channel (A), agricultural drainage channel after cleaning from algae and wastes (B)

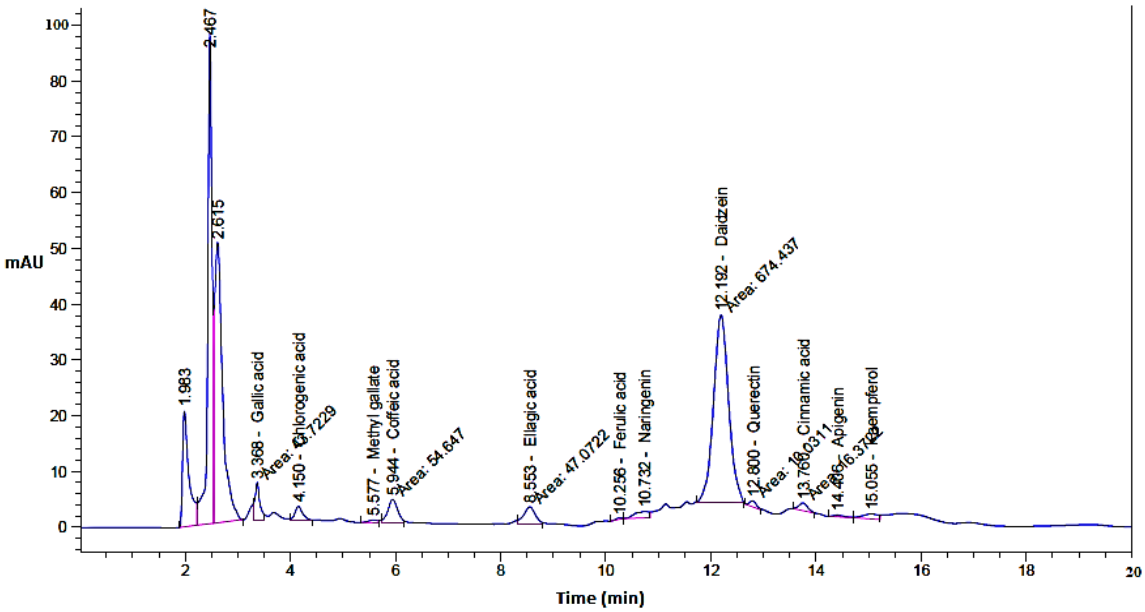

Fig. 2. Chromatograms of detected phenolic and flavonoid constituents *via* HPLC

Table 1. Phenolic and Flavonoid Constituents of Algal Biomass

Constituent Name	Retention Time	Area (%)	Area (%)	Concentration (µg/mL)
Unknown	1.983	176.88	8.21	Undetected
Unknown	2.467	518.03	24.04	Undetected
Unknown	2.615	537.62	24.95	Undetected
Gallic acid	3.368	43.72	2.03	233.29
Chlorogenic acid	4.150	24.28	1.13	205.18
Catechin	4.622	0.00	0.00	0.00
Methyl gallate	5.577	5.59	0.26	18.89
Caffeic acid	5.944	54.65	2.54	262.45
Syringic acid	6.611	0.00	0.00	0.00

Pyro catechol	6.808	0.00	0.00	0.00
Rutin	8.032	0.00	0.00	0.00
Ellagic acid	8.553	47.07	2.18	596.08
Coumaric acid	9.209	0.00	0.00	0.00
Vanillin	9.808	0.00	0.00	0.00
Ferulic acid	10.256	1.89	0.09	7.93
Naringenin	10.732	21.37	0.99	155.75
Daidzein	12.192	674.44	31.29	2554.78
Quercetin	12.800	10.03	0.47	80.77
Cinnamic acid	13.760	16.37	0.76	18.62
Apigenin	14.406	4.89	0.23	22.62
Kaempferol	15.055	18.39	0.85	81.76
Hesperetin	15.636	0.00	0.00	0.00

As the algal extract contains numerous compounds of phenolic and flavonoids, as well as secondary metabolites, its ability to reduce copper compounds to nanoparticles increased. The main reducing agent in algae extract for CuONPs creation may be the compounds that possess several functional groups such as chlorogenic acid and quercetin. Therefore, the algal biomass was subjected to CuONPs synthesis. UV-visible spectroscopy showed the maximum absorption peak at 250 nm. Their surface plasmon resonance at different temperatures indicated that algal biomass was able to create CuONPs at elevated conditions of temperature (Fig. 3). The UV spectrum was compared to algal biomass without the addition of copper salt. The authors' observation agrees with the other studies on the creation of CuONPs by algae, which shows that the CuONPs peak was at 247 nm (Mohamed *et al.* 2021a). The size and morphology of created CuONPs were characterized by TEM (Fig. 4). CuONPs appeared in spherical form (average size 5 to 17 nm). The dispersed NPs were surrounded with capping by algal active metabolites. Mohamed *et al.* (2021a) noted that the size range of created CuONPs was 21.8 nm. In this context, brown algae *Macrocystis pyrifera* was mediated for CuONPs creation within a size of 2 to 50 nm (Araya-Castro *et al.* 2021).

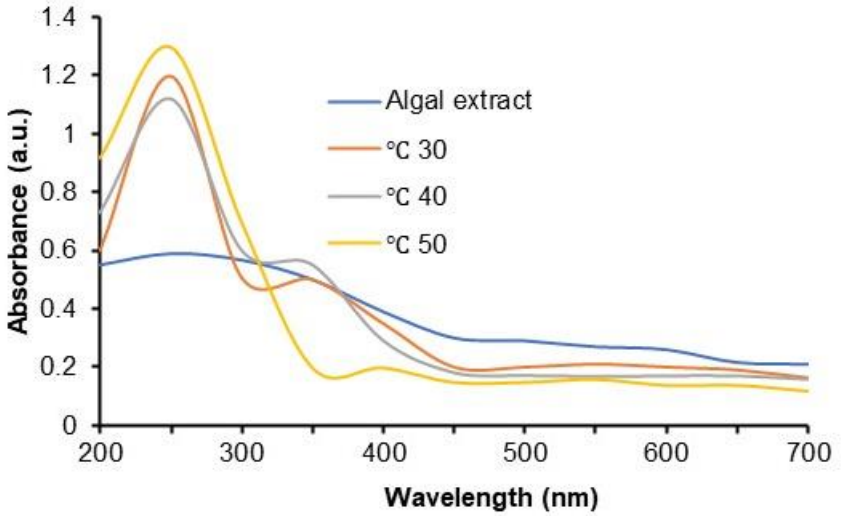

Fig. 3. Absorbance peaks of the green synthesized CuONPs using a UV - Vis spectrophotometer

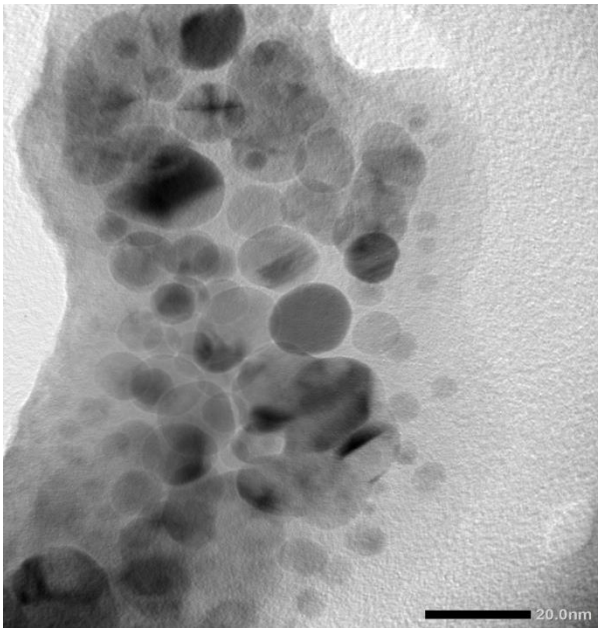

Fig. 4. TEM micrograph of created CuONPs by algal biomass

The crystalline structure of CuONPs was validated using XRD analysis, as shown in Fig. 5. The primary strong angles in the diffractogram of biosynthesized CuONPs were visible in the XRD patterns, showing that CuONPs were crystallographic in nature (Mohamed *et al.* 2021b; Hammad *et al.* 2022). Figure 1 shows XRD diffraction peaks of CuONPs, and displays the diffraction characteristics regarding 2θ at 33.8°, 35.5°, 37.9°, 48.1°, 51.6°, 61.01°, and 66.2°, which represented the Bragg's reflections at 110, 111, -111, -202, 020, -113, and 022, respectively. The mineral crystal was CuO of the tenorite crystal form. Thus, all of the observed peaks were similar to those reported by the joint committee on powder diffraction standards (JCPDS) of CuO-NPs with a standard card JCPDS File No: 01-1117, as recorded by Badawy *et al.* (2021), and Shehabeldine *et al.* (2023). Therefore, the results clearly support the CuONPs synthesis. The CuONPs diffractogram does not reveal the presence of any other impurities, ensuring that the CuONPs obtained were pure and in agreement with other investigations (Shende *et al.* 2015; Nabila and Kannabiran 2018; Mohamed *et al.* 2021).

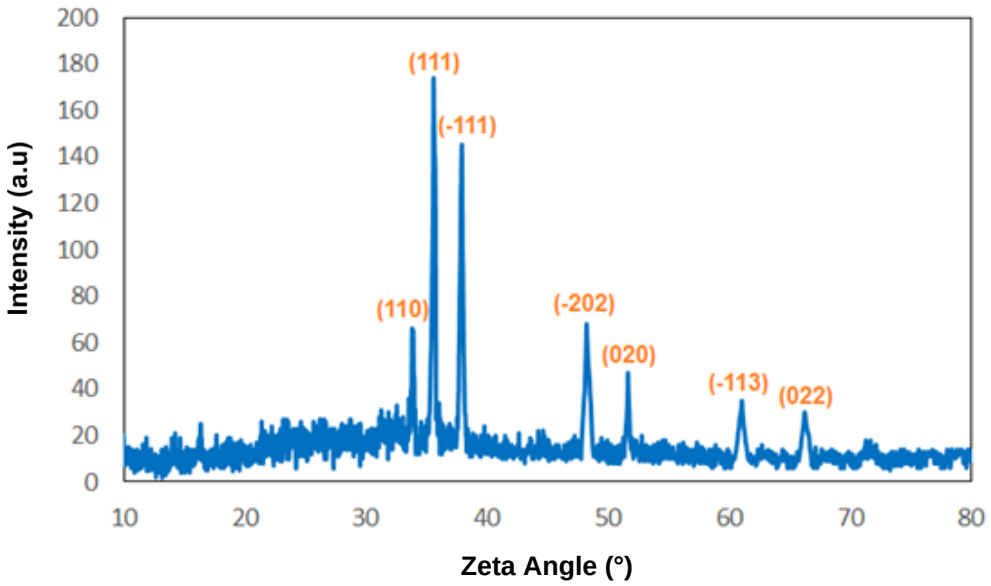

Fig. 5. XRD pattern of the biosynthesized CuONPs

Copper compounds have been previously applied as fertilizer and fungicide, and several studies recommend that CuONPs up to certain concentrations are not toxic and efficiently repress disease development in plants (Faraz *et al.* 2022). The current investigation reflected the impact of CuONPs on phytopathogenic (*C. lunata* and *F. oxysporium*), mycotoxigenic (*A. flavus* and *A. terreus*), and bio-applicable (*M. anisopliae* and *T. harzianum*) fungi compared to copper acetate, algal extract, and chemical fungicide (Table 2). The growth of all tested fungi decreased with increasing concentration of CuONPs and fungicide, where at 50 mg the colony diameter was 4.02 ± 0.08, 5.92 ± 0.11, 4.50 ± 0.08, 7.10 ± 0.04, 4.78 ± 0.04, and 3.87 ± 0.03 cm; while at 200 mg the colony diameter was 1.84 ± 0.08, 1.97 ± 0.03, 1.00 ± 0.08, 2.04 ± 0.03, 2.32 ± 0.06, and 2.42 ± 0.05 cm of *M. anisopliae*, *T. harzianum*, *C. lunata*, *F. oxysporium*, *A. flavus*, and *A. terreus*, respectively. The chemical fungicide showed more inhibitory action than CuONPs. In contrast, algal extract exhibited stimulatory action for growth of all tested fungi at 50 and 100 mg, but at high concentration, 200 mg showed negligible fungal inhibition compared with the control.

Table 2. Effect of Different Concentrations of Copper Acetate, Algal Biomass, CuONPs and Chemical Fungicide on Fungal Growth

Concentration (mg)	*M. anisopliae* Colony Radius (cm)				*T. harzianum* Colony Radius (cm)			
	Copper Acetate	Algal Biomass	CuONPs	Chemical Fungicide	Copper Acetate	Algal Biomass	CuONPs	Chemical Fungicide
0	6.78 ± 0.18a	6.78 ± 0.18a	6.78 ± 0.18a	6.78 ± 0.10a	7.80 ± 0.03a	7.80 ± 0.03a	7.80 ± 0.03a	7.80 ± 0.14a
50	6.81 ± 0.07a	6.79 ± 0.05a	4.02 ± 0.08b	3.10 ± 0.07b	7.84 ± 0.07a	7.84 ± 0.06a	5.92 ± 0.11b	4.50 ± 0.04b
100	4.12 ± 0.05b	6.80 ± 0.05a	2.20 ± 0.10c	1.25 ± 0.05c	5.87 ± 0.05b	7.82 ± 0.03a	4.62 ± 0.05c	2.22 ± 0.08c

200	2.02 ± 0.06c	5.48 ± 0.12b	1.84 ± 0.08d	0.80 ± 0.02d	3.42 ± 0.05c	5.54 ± 0.07b	1.97 ± 0.03d	0.87 ± 0.05d
Concentration (mg)	***C. lunata* Colony Radius (cm)**				***F. oxysporium* Colony Radius (cm)**			
0	6.20 ± 0.10a	6.20 ± 0.10a	6.20 ± 0.18a	6.20 ± 0.10a	8.08 ± 0.06a	8.08 ± 0.06a	8.08 ± 0.06a	7.98 ± 0.18a
50	5.10 ± 0.07b	6.35 ± 0.09a	4.50 ± 0.08b	2.48 ± 0.08b	7.70 ± 0.12b	8.10 ± 0.05a	7.10 ± 0.04b	4.38 ± 0.02b
100	3.73 ± 0.05c	6.30 ± 0.04a	2.20 ± 0.10c	1.18 ± 0.10c	5.43 ± 0.05c	7.15 ± 0.04b	5.08 ± 0.05c	2.10 ± 0.11c
200	1.95 ± 0.03d	4.88 ± 0.09b	1.00 ± 0.08d	0.78 ± 0.05d	4.00 ± 0.04d	6.90 ± 0.06c	2.04 ± 0.03c	1.50 ± 0.05d
Concentration (mg)	***A. flavus* Colony Radius (cm)**				***A.** terreus* **Colony Radius (cm)**			
0	6.54 ± 0.03a	6.54 ± 0.03a	6.54 ± 0.03a	6.52 ± 0.03a	4.08 ± 0.14a	4.06 ± 0.14a	4.08 ± 0.14a	4.08 ± 0.14a
50	6.58 ± 0.04a	6.88 ± 0.09b	4.78 ± 0.04b	4.00 ± 0.02b	3.87 ± 0.04b	4.34 ± 0.03b	3.10 ± 0.03b	2.50 ± 0.05b
100	3.46 ± 0.05b	6.82 ± 0.03b	3.46 ± 0.03c	2.42 ± 0.05c	3.17 ± 0.05b	4.28 ± 0.04b	2.98 ± 0.03b	2.10 ± 0.06c
200	2.88 ± 0.08c	5.82 ± 0.06c	2.32 ± 0.06d	1.33 ± 0.05d	2.74 ± 0.03c	4.10 ± 0.06a	2.42 ± 0.05c	1.00 ± 0.03d

Growth of three fungi, including *M. anisopliae, T. harzianum,* and *A. flavus*, were encouraged with low concentration of 50 mg of copper acetate, where the colony growth was 6.81 ± 0.07, 7.84 ± 0.07, and 6.58 ± 0.04 cm compared to colony growth 6.78 ± 0.18, 7.80 ± 0.03, and 6.54 ± 0.03 cm for the control, respectively. The colony radius of *C. lunata*, *F. oxysporium,* and *A.terreus* decreased with increasing copper acetate. Finally, the inhibitory action against tested fungi was attributed to the chemical fungicide followed by CuONPs, followed by copper acetate, and algal extract. Banik and Pérez-de-Luque (2017) investigated the influence of CuONPs against phytopathogenic and beneficial microorganisms; they observed that CuONPs did not significantly inhibit *Trichoderma harzianum* and *Rhizobium* spp. compared to chemical fungicide (copper oxychloride). Additionally, they showed that the growth of *Fusarium oxysporum, T. harzianum, Botrytis fabae, Alternaria alternate,* and *Pseudomonas syringae* was promoted at low concentration of CuONPs. CuONPs interfere with germination of fungal spores *via* affecting the metabolic pathways of fungi (Gaba *et al.* 2022). Arya *et al.* (2018) documented the antibacterial and antifungal potential of created CuONPs by green alga *Botryococcus braunii* against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Escherichia coli*, and *Fusarium oxysporum*. Recently, Atri *et al.* (2023) showed that CuONPs reflected efficient antifungal activity.

Moreover, the antibacterial activity of CuONPs was recorded compared to to copper acetate, algal extract, and standard antibiotic (Fig. 6). CuONPs exhibited promising inhibitory action against all tested bacteria with inhibition zones of 26, 23, 25, and 22 mm compared to antibiotic with inhibition zones of 21, 22, 21, and 18 mm against *B. subtilis, E. coli, K. pneumoniae,* and *S. aureus,* respectively. Copper acetate also showed antibacterial activity against all tested bacteria but less CuONPs and antibiotic. Negligible inhibition zones were observed using algal biomass extract against only *B. subtilis,* and *E. coli.* According to Nabila and Kannabiran (2018), the green created CuONPs by actinomycetes revealed bacteriostatic potential against various bacterial pathogens for human and fish including *Bacillus* cereus, *Edwardsiella tarda, S. aureus, Proteus*

mirabilis, *Vibrio anguillarum*, *Aeromonas hydrophila*, and *A. caviae*. Copper metal (Cu^{2+}) exhibits activity against bacteria and is accepted by US-EPA (US-Environmental Protection Agency) as a harmless agent for fighting microorganisms. The CuONPs have more antibacterial activity compared to the non-nanoform of copper compound (Vasantharaj *et al.* 2023).

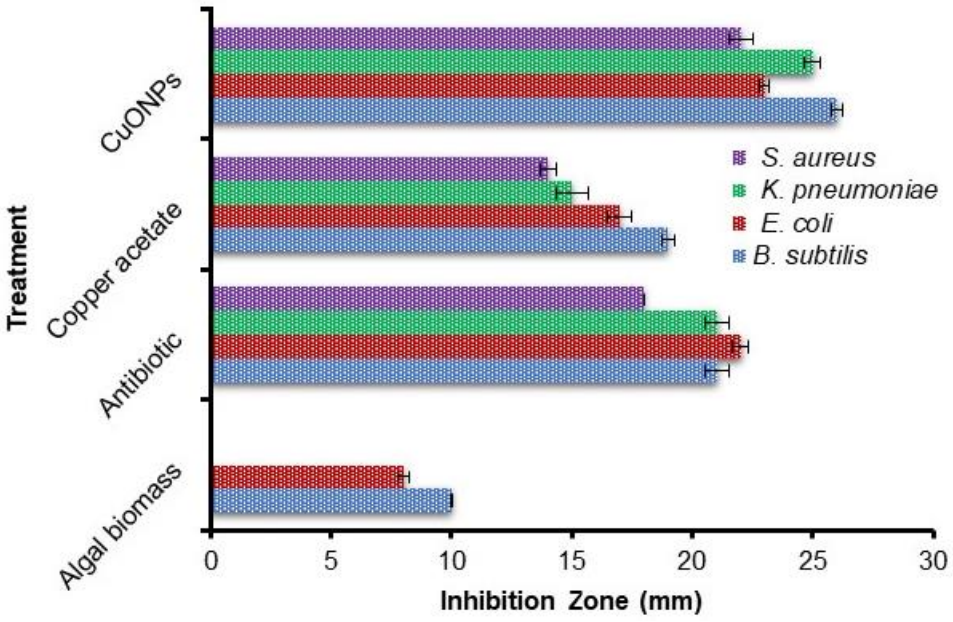

Fig. 6. Antibacterial activity of copper acetate, algal biomass, CuONPs, and antibiotic

Degradation quantity of methyl green and methyl orange dyes increased with the increasing time as a result of exposure to CuONPs as a photocatalyst (Fig. 7). At 90 min, the degradation quantity was 75.67 ± 0.58 and 64.67 ± 3.21% for methyl green and methyl orange dyes, respectively. There was negligible increase in degradation quantity of dyes after 75 min, where degradation quantity was 75.33 ± 1.53 and 63.67 ± 1.53% for methyl green at 75 min, while it was 75.67 ± 0.58 and 64.67 ± 3.21% at 90 min. If the exposure time was augmented, more efficiency of dyes degradation could be obtained. Degradation quantity of methyl orange was less than methyl green at all different times. Degradation of dyes by CuONPs was recorded in other studies but with different levels depending on many factors, such as dyes concentration as mentioned by Aroob *et al.* (2023), where 20 ppm of methyl green and methyl orange dyes showed less degradable compared to 10 ppm. Sharma and Sharma (2017) showed that methyl orange was degraded up to 96% *via* green synthesized CuONPs in the existence of UV light at 24 min, while Ikram *et al.* (2022) showed degradation efficiency was 45.2% and 32.0% *via* green synthesized CuONPs in the presence of UV light and sunlight, respectively, at 60 min. Atri *et al.* (2023) revealed that the green synthesized CuONPs can play a vital role in the degradation of dyes in industrial and domestic waste.

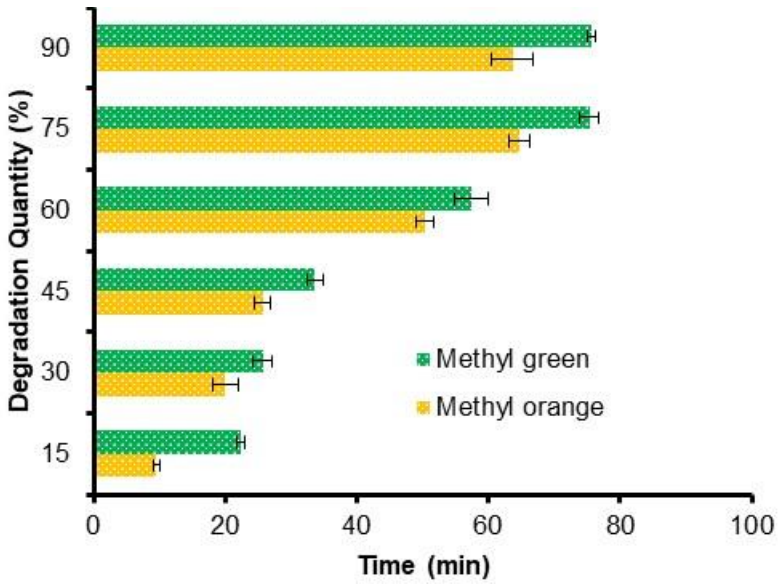

Fig. 7. Degradation quantity of methyl green and methyl orange by CuONPs at different times

CONCLUSIONS

1. The available algae in the agricultural drainage channel were exploited in the green synthesis of copper oxide nanoparticles (CuONPs), and these NPs were applied to combat phytopathogenic and mycotoxigenic fungi, and their activity against fungi exploited in biotechnology was also evaluated compared to the effect of a chemical fungicide on all tested fungi.
2. The created CuONPs, which were identified as tenorite based on X-ray diffraction (XRD), effectively revealed photocatalytic activity to remove methyl orange and methyl green degradation.
3. The obtained promising findings offered attractive resources for investigators to produce economical and eco-friendly fungicide and photocatalyst to control fungal development and efficiently reduce dyes water contamination.

Funding

This work was supported and funded by the Deanship of Scientific Research at Imam Mohammad Ibn Saud Islamic University (IMSIU) (grant number IMSIU-RP23038), Riyadh, Saudi Arabia.

Acknowledgments

The authors wish to appreciate the Deanship of Scientific Research at Imam Mohammad Ibn Saud Islamic University (IMSIU) for support and funding the current study (grant number IMSIU-RP23038)

Conflicts of Interest

The authors declare no conflict of interest.

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Article submitted: July 29, 2023; Peer review completed: September 9, 2023; Revisions received: September 9, 2023; Accepted: September 10, 2023; Published: September 18, 2023.
DOI: 10.15376/biores.18.4.7474-7489