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Original Research

Examination of the Antifungal Potential of Ozone Versus *Aspergillus Parasiticus* Isolated from Water

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Abstract

The ecosystem and human health are being put at greater risk by the fungal pollution of water sources. In this study, the inactivation of the most common fungal isolate in water sources using ozone was reported. Six fungal isolates were isolated from Al-Azhar tap water, Al-Azhar wastewater, El-Menia canal water, and the El-Menia water treatment plant, where *Aspergillus parasiticus* (*A. parasiticus*) was the most common fungal isolate in all tested water samples. The fungi were morphologically identified, and *A. parasiticus* identification was confirmed using molecular identification. The variation of the dry weight of the isolated fungal species was reported upon exposure to different doses of ozone ranging from 10 µg/cc to 60 µg/cc, where the dry weight of *A. parasiticus* was the first fungal isolate that was significantly ($P \leq 0.05$) reduced upon using 10 µg/cc. The impact of using various doses of ozone for 5 minutes in metals levels on both Al-Azhar wastewater and El-Menia water treatment plant was compared versus using (50, 100, and 200 ppm) of chlorine, reflecting that using 10 µg/cc of ozone for 5 minutes could be applied as an effective dose in decreasing levels of metals. Furthermore, secretion of aflatoxins by *A. parasiticus* was dramatically decreased ($P \leq 0.05$) upon exposing the fungus to 10 µg/cc ozone for 5 minutes relative to untreated fungus. The present results revealed the possibility of using a low dose of ozone to decrease the growth of fungal pathogens in water and their possible secreted toxins.

Keywords: fungi, ozone, *Aspergillus parasiticus*, heavy metals, flatoxins

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Introduction

It is essential to prevent the spread of infections from contaminated objects and surfaces through disinfection using various agents, including ozone, which offers significant environmental and financial advantages that guarantee the reuse of various resources through waste management and expenditure reduction [1]. Ozone is an inorganic molecule that has strong antimicrobial capabilities because of its third oxygen atom, which is loosely bound and easily oxidizes other compounds. Numerous studies have shown that it negatively affects the cell membrane, unsaturated fatty acids, essential proteins, DNA, and enzymes inside the cells of microbes [2-5]. Ozone is one of the few disinfectant substances that may be used for disinfection in both gaseous and aqueous forms, which is one of the reasons for its broad application when compared to other commonly used sanitizing chemicals. Furthermore, ozone is unstable and eventually breaks down into oxygen on its own [6-8]. Some information on various configurations used to utilize aqueous ozone for commercial cleaning applications [9, 10].

A comprehensive evaluation of treatment options using ozone is inadequate in the literature, despite the fact that multiple studies have independently assessed the impact of ozone's antimicrobial characteristics in both air and water employing a range of microorganisms [11-13]. The Centers for Disease Control (CDC) states that further studies are necessary to determine the efficacy of ozone mists in reducing pollutants in the environment [14, 15]. Depending on the exposure period and O_3 concentration, fungi may exhibit varying degrees of sensitivity and tolerance [16]. The substrate, spore shape, and moisture content all have an impact on sensitivity/tolerance. When exposed to gaseous O_3, spore inactivation and mycelial growth suppression have varied in their efficacy. For instance, Vijayanandraj et al. [17] showed that O_3 treatment did not influence spore germination but did lower *Aspergillus niger* mycelial growth.

The effectiveness of gaseous O_3 treatment as a fungicidal or fungistatic agent has been disputed [18]. According to Mylona et al., *Fusarium verticillioides* conidia exposure to 200-300 ppm O_3 exposure for one hour was initially successful, but over the course of the next 10 days, spore viability recovered and did in fact lead to the synthesis of fumonisins under various circumstances. Although the effect of gaseous O_3 on spore viability has been studied, it is likely necessary to pay greater attention to the capacity for physiological repair [19]. The purpose of this study was to investigate the ability of various gaseous O_3 levels to decrease or suppress populations of fungi isolated from various water sources, particularly *Aspergillus parasiticus*, a fungus that was frequently isolated. Following treatment, the mycotoxins contamination was identified utilizing sub-lethal ozone levels.

Materials and Methods

Ozone Source

Ozone was produced using an ozonizer (Ozo mammoth T936, Egypt) from oxygen gas. Ozone generator with changeable pure oxygen flow meter rates of 1/2, 1/4, 1/8, 1/16, and 1/32 LPM intended to create precise variable concentrations. The test samples were placed in a collecting flask that was submerged in a cold bath to maximize ozone solubility. A Teflon tube was used to transport the ozone created by the generator into the specimens.

Specimens

Four water samples were used as the source for the fungal isolates, including tap water from Al-Azhar University (30.0580°N, 31.3126°E), wastewater (30.0480°N, 31.4128°E) from Al-Azhar University, water from the El Menia Canal in Samallot City (28,11944°N,30.74444°E), and an outlet from the El Menia Water Treatment Plant in Samallot City (28.3140°N, 30.7101°E).

Isolation, Purification, and Identification of Fungal Isolates

One liter of each sample was filtered through a 0.45 mm filter, which was transferred to sterile plates containing malt agar medium and gentamicin (250 µg/L). The plates were cultured for 48 hours at 28°C for fungal isolates. Individual colonies on agar medium were picked up, streaked on another prepared agar plate for each colony to ensure gaining pure isolates and incubated for the same conditions. Through the use of Soft-Imaging GmbH software (ANALYSIS Pro ver. 3.0, Germany) on an image analysis system (Olympus BX 40), the fungal isolates were put through several morphological examinations. For the molecular identification: Using the Quick-DNA Fungal Microprep Kit (Zymo Research; D6007) and the assistance of Sigma Scientific Services Company (Egypt), DNA was extracted from agar cultures in accordance with the manufacturer's instructions. Primer A, 5′-CATGCTCCATCATGGTGACT-3; and Primer R, 5′-CCGCCGCTTTGATCTAGG-3 were both used. A phylogenetic tree was created using ITOL software [20].

Determination of the Lowest Ozone Dosage Inhibits Fungal Growth

A serial dilution of fungal isolates starting from ($5X10^4$ colony-forming-units [CFU] per mL) was prepared. Quantitative tests were carried out, each consisting of 100 µL drops of suspension, in duplicates, dried onto plastic trays (usually the underside of the lid of a micro well plate), under sterilized conditions,

stirring for homogenization. 50 ml of broth media for each of the malt agar media were inoculated with 1 mL of spore suspension. All microbial flasks were treated with six different doses (10-20-30-40-50-60 μg/cc), which were the lowest and highest doses of the ozone generator used in this study, for five minutes of exposure to 90-99.9% relative humidity at room temperature using the ozonizer. Three replications were reported and compared with the control. The incubation time was 28ºC for 7 days. Fungal growth was measured by dry weight after mycelium filtration and drying at 40-60ºC [21].

Comparison Between the Lowest Dose of Ozone and Biocidal Disinfection Agent as Chlorine

Two sources of samples were used for the study of the action of ozone for water purification: Al Azhar wastewater and the El Menia water treatment plant were tested in this comparison between ozone and chlorine. A different dose (10-20-30-40-50-60 mg/cc) of ozone was applied in one liter of water sample from the two sources. Besides, solution (6%) ultra-germicidal bleach was made into three different sodium hypochlorite concentrations. To achieve a final concentration of 50, 100, or 200 ppm, sterile water was combined with sodium hypochlorite and applied to the two representative samples (during the course of 10 minutes). One ml of each treated sample was submitted to X-ray (JEOL, Japan) to determine the percentage of heavy metals [22].

Effect of Ozone on the Ultrastructures of the Most Sensitive Microorganisms

The most common fungal isolates were subjected to study their morphological and ultrastructure changes upon treatment with ozone.

For Scanning Electron Microscopy (SEM): Blocks of the investigated fungal isolate were prepared and examined for SEM at the National Research Centre, Dokki, Cairo, Egypt. Six to eight millimeter squares of agar containing fungal growth were cut from the cultures for fixation and dehydration treatments using the programmed (LEICA EM TP, Germany) tissue processor model (A-1170). The squares were then fixed by submerging them in 2% (w/v) aqueous osmium tetroxide (OsO_4) for 12 hours at 4°C. In order to eliminate excess OsO_4, the fixed material was allowed to reach room temperature before being rinsed three times in distilled water for ten minutes each. Materials that had been fixed and cleaned were immersed and dried using a succession of ethanol concentrations that ranged from 10% to 90% and then 100% ethanol. Using pressure, critical point drying was performed on dehydrated specimens. Then, using a carbon adhesive, the critical point-dried specimens were fastened on 0.9 mm diameter copper stubs. The samples were coated in gold by Polar Instruments Inc., Doylestown, Pennsylvania (almost 50 nm thickness) and then inspected with a (JEOL JSM-35LV Scanning Electron Microscope, Japan) in high-vacuum mode [23].

For transmission electron microscopy (TEM): One mm^3 block of the tested isolate was embedded in 2% agar and dehydrated using a graduated series of ethanol before being fixed with 3% glutaraldehyde, 1% paraformaldehyde, and 1% osmium tetroxide at 4ºC over the course of an overnight period. Living cells were put between two copper discs for fast freezing and freeze-substitution, which involved swiftly freezing the cells by submerging them in propane slush in liquid nitrogen and freeze-substituting them for two days in acetone with 2% osmium tetroxide at -80ºC. For freeze substitution after glutaraldehyde fixation, cells were fixed in a mixture of 3% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at room temperature for 30-60 min. or at 4ºC overnight. They were collected by centrifugation, rapidly frozen by propane slush, and freeze substituted in acetone containing 2% osmium tetroxide at 80ºC for 2 days. They were then implanted in epoxy resin and polymerized at 6ºC for 24 hours using these variously fixed and dehydrated samples. On an ultra-microtome (Leica ultracut, Germany), ultra-thin sections were cut to a thickness of 70 to 90 nm and put on copper grids. At the National Research Centre, Dokki, Cairo, Egypt, they are dyed with uranyl acetate and lead citrate, coated with plasma-polymerized naphthalene support film, and studied at 80kv in a JEM 12000EX TEM (JEOL, Japan) [24].

Application of Ozone in Reduction of Aflatoxin Produced by *A. Parasiticus.*

Extraction of Aflatoxins

Inoculation of one ml of spore suspension (10^4 cells/ml) of *A. parasiticus* in 250 ml Erlenmeyer flasks each containing 100 ml of sterile yeast extract agar (YES) broth medium treated with ozone at 10 μg/cc for five minutes. Three replicates for each concentration were prepared and incubated at 25.0±2.0ºC for 21 days. The broth filtrates were mixed with an equal volume of chloroform in a separating funnel. The residue was re-extracted twice for complete extraction. The chloroform extract was defatted with hexane for a separate lipid layer and concentrated in a rotary evaporator. The residues were reconstituted in one ml of methanol for further chromatographic analysis [25].

Determination of Aflatoxins B1, B2, G1, and G2 by HPLC

To evaluate the detoxifying power of ozone gas, the concentrations of aflatoxins in control and treated samples were analyzed for the quantification of aflatoxins using immunoaffinity columns supplied by Rhône Diagnostics Technologies Ltd. (Spain) and quantified by high-performance liquid chromatography (HPLC). The solvent mixture was water: methanol (8:2) [26].

The sample extract was filtered, diluted, and applied in an immunoaffinity column containing antibodies specific to aflatoxins B1, B2, G1, and G2. Standard aflatoxins (AF) B1, B2, G1, and G2 were purchased from Sigma-Aldrich (Ref.A-6636, A-9887, A-0138, and A-0263, respectively) (Quimica S.A. Spain).

Statistical Analysis

Three replicates were recorded, data were represented as means, and a t-test was applied using Graphpad Prism Software V.5 (CA, USA) to calculate the difference.

Results

Identification of Fungal Isolates

Various water sources contained different fungal isolates, where Al-Azhar tap water contained the highest number of different fungal isolates (4), while the El-Menia water treatment plant contained the lowest number of fungi (1), as shown in (Table 1).

The fungi were subjected to morphological examination for identification of the six different fungal isolates as follows:

Isolate no. (1): Radiate conidial heads; conidiophore contained stips smooth-walled hyaline or pigmented. Vesicles were subspherical, 55-99 µm diam. conidiogenous cells biserate, and the Metulae were twice as long as the phialides. Conidia were brown, ornamented with warts and ridges, sub-spherical, 3.5-5.0 µm in diameter, which identified as *Aspergillus niger* as shown in (Fig. 1).

Isolate No. (2) contained conidial heads densely columnar. conidiophore stipes smooth–walled, hyaline. vesicles subspherical, 9-19 µm diam. conidiogenous cells bi-seriate. Metulae were as long as the phialides. Conidia were smooth-walled, striate, spherical to broadly ellipsoidal, 1.3-2.7 µm, hyaline, which was identified as *Aspergillus terreus* as shown in (Fig. 1).

Isolate no. (3) contained conidiophore stipes 65-300 µm long, smooth-walled, hyaline, conspicuously encrusted, biverticillate. Metulae and phialides 10-14 µm long. Phialides were acerose. conidia ellipsoidal, sometimes sub-spherical apiculate, irregularly roughened, 3.0-3.5×2.5-3.0 µm, which is identified as *Penicillium purpurogenum* as shown in (Fig. 1).

Isolate no. (4) Conidia were obclavate to ellipsoidal, with a short, cylindrical beak, with dimensions 22-55 X 7-18 µm, medium brown, rugolose with muriform septation, with a single scar at the tip, arising in mostly unbranched chains of ten or more, which were identified as *Alternaria alternate* as shown in (Fig. 1).

Isolate no. (5) contained conidiophores erect, short, strongly branched, and strongly geniculate with conidia on the nodes. Conidial scars: hyaline. Single conidia in very short chains, obovoidal, without beaks, medium brown to oloivaceous verrucose with dimensions of 20-25 X 8-12 µm, with 1-3 transverse and 0-2 oblique or longitudinal septa, which are identified as *Ulocladium botrytis* as shown in (Fig. 1).

Isolate no. (6) contained conidial heads consistently and loosely radiating, up to 400-500 µm in diameter; conidiophores variable in length, from 200µ to rarely more than 1.0 mm, mostly 300-700 µ long, with walls colorless, smooth, or nearly so in some strains identified as *Aspergillus parasiticus,* as shown in (Fig. 1).

It was observed that isolate no. (6) was the most common isolate that could be isolated from all sources, as shown in (Table 2) and subjected to molecular identification, and the identified isolate was deposited in the gene bank with accession number OR511577.1, https://www.ncbi.nlm.nih.gov/nuccore/OR511577.1, and the phylogenetic tree showed the high similarity with related species as shown in (Fig. 2).

Table 1. Number of the fungal isolates from the different sources (Data are represented as means ± SD).

Sources	Isolate no. / Source type	Number of fungal isolates
Water	Al-Azhar tap water	4±1
	Al-azhar waste water	2±1
	El-Menia canal water	2±1
	El-Menia water treatment plant	1±1

Determination of Ozone Impact on Fungal Inhibition

It could be noticed that there was a gradual decrease in dry weight of isolated fungi, where *A. parasiticus* was the most affected fungal isolate after exposure for 5 minutes at room temperature, followed by *Alternaria alternate* and *Aspergillus niger*. A significant decrease in the dry weight of *A. parasiticus* ($p \leq 0.05$) upon using 10 µg/cc of ozone as shown in (Table 3). Besides, *Aspergillus terrus, Aspergillus niger, and Penicillium purpurogenum* have a dramatic decrease ($p \leq 0.05$) in their dry weight upon exposure to 40 µg/cc. Additionally, *Alternaria alternate* has a dramatic decrease ($p \leq 0.05$) in its dry weight upon exposure to 20 µg/cc of ozone. Lastly, *Ulocladium botrytis* has a dramatic decrease ($p \leq 0.05$) in dry weight upon exposure to 50 µg/cc as shown in (Table 3).

Impact of Using Ozone and Chorine on Heavy Metals

Al Azhar wastewater and the El Menia water treatment plant were selected as the highest and lowest water sources that contained the highest and the lowest number of fungal isolates. Exposure of sewage water

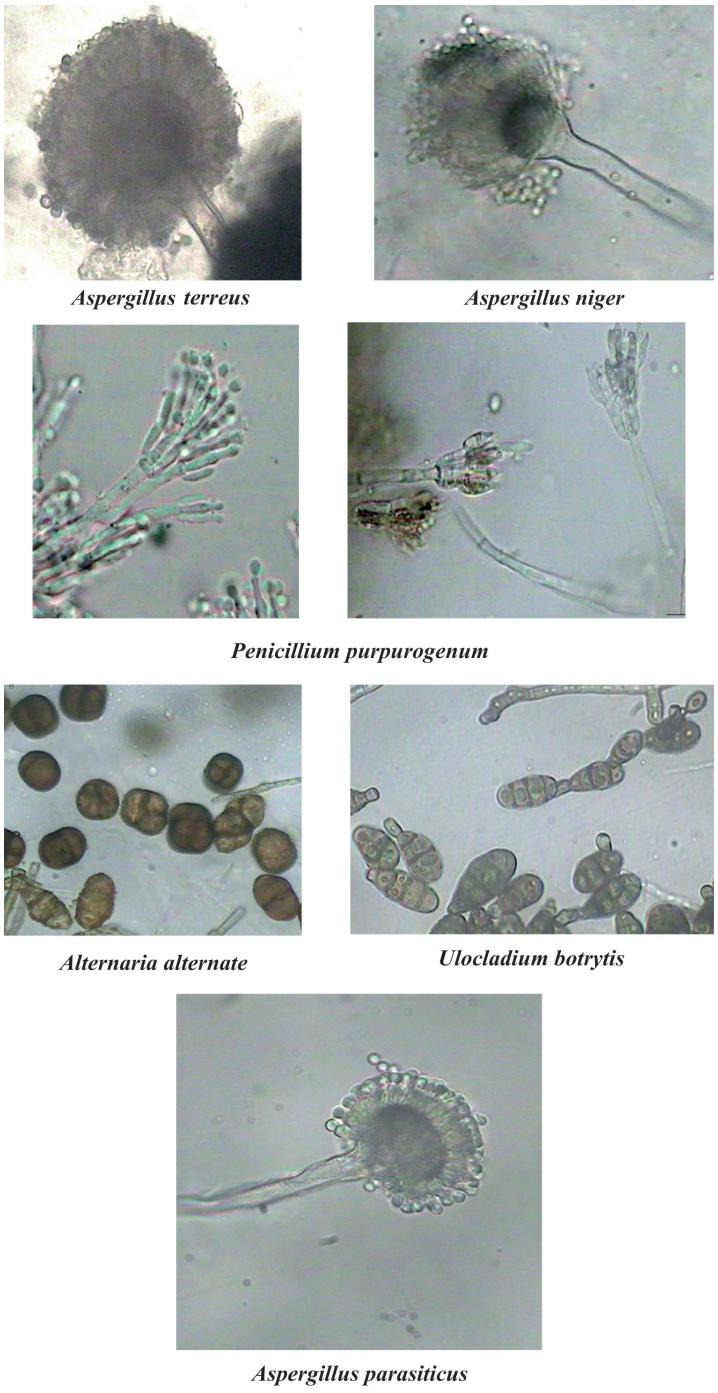

Fig. 1. Different identified fungal isolates isolated from different water sources (Magnification 40X).

and water treatment plant to ozone concentration starting from 10 µg/cc at 5 minutes showed a significant reduction in heavy metals ($p \leq 0.05$) compared to the control. Exposure to chlorine at 100 ppm for 10 min has a significant impact on all tested heavy meals relative to control ($p \leq 0.05$) as shown in (Table 4).

Effect of Ozone on the Ultra-Structures of the Most Sensitive Fungal Isolates

The ozone showed damage and vital effects on fungal structures upon examination using transmission and scanning electron micropscopes, whereas cellular shape distorted and became smaller and abnormal, where organelles lysed and experienced shrinkage of the cytoplasm membrane upon exposure to 10 µg/cc for 5 minutes for *A. parasiticus, as* shown in (Fig. 3).

Effect of Ozone on Qualitative and Quantitative Production of Aflatoxins

A. parasiticus produces aflatoxins (G1, G2, B1, and B2). Ozone showed a good reduction of the production of all aflatoxins by *A. parasiticus* after being treated with

Table 2. Identification of the fungal Isolates from the different sources.

Source	Fungal isolates
Al-Azhar tap water	*Aspergillus terrus.* *Aspergillus parasiticus* *Penicillium purpurogenum* *Alternaria alternate* *Aspergillus parasiticus* *Aspergillus niger*
Al-azhar waste water	*Aspergillus niger* *Aspergillus parasiticus*
El-Menia canal water	Ulocladium botrytis *Aspergillus parasiticus*
El-Menia water treatment plant	*Aspergillus parasiticus*

ozone at 10 µg/cc for 5 minutes as compared with the control. Samples had been submitted for analysis of the mycotoxin contents. There was a significant reduction ($p \leq 0.05$) in the production of Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, and Aflatoxin G2 by quantitative analysis using HPLC as shown in (Fig. 4, Table 5).

Discussion

Ozone is a powerful oxidizer that can be utilized in a variety of industries as a disinfectant at low concentrations and for limited contact times [27-29]. The safety of drinking water has been put at risk by the widespread spread of the fungus, which has received a lot to focus on during the process of treatment of water

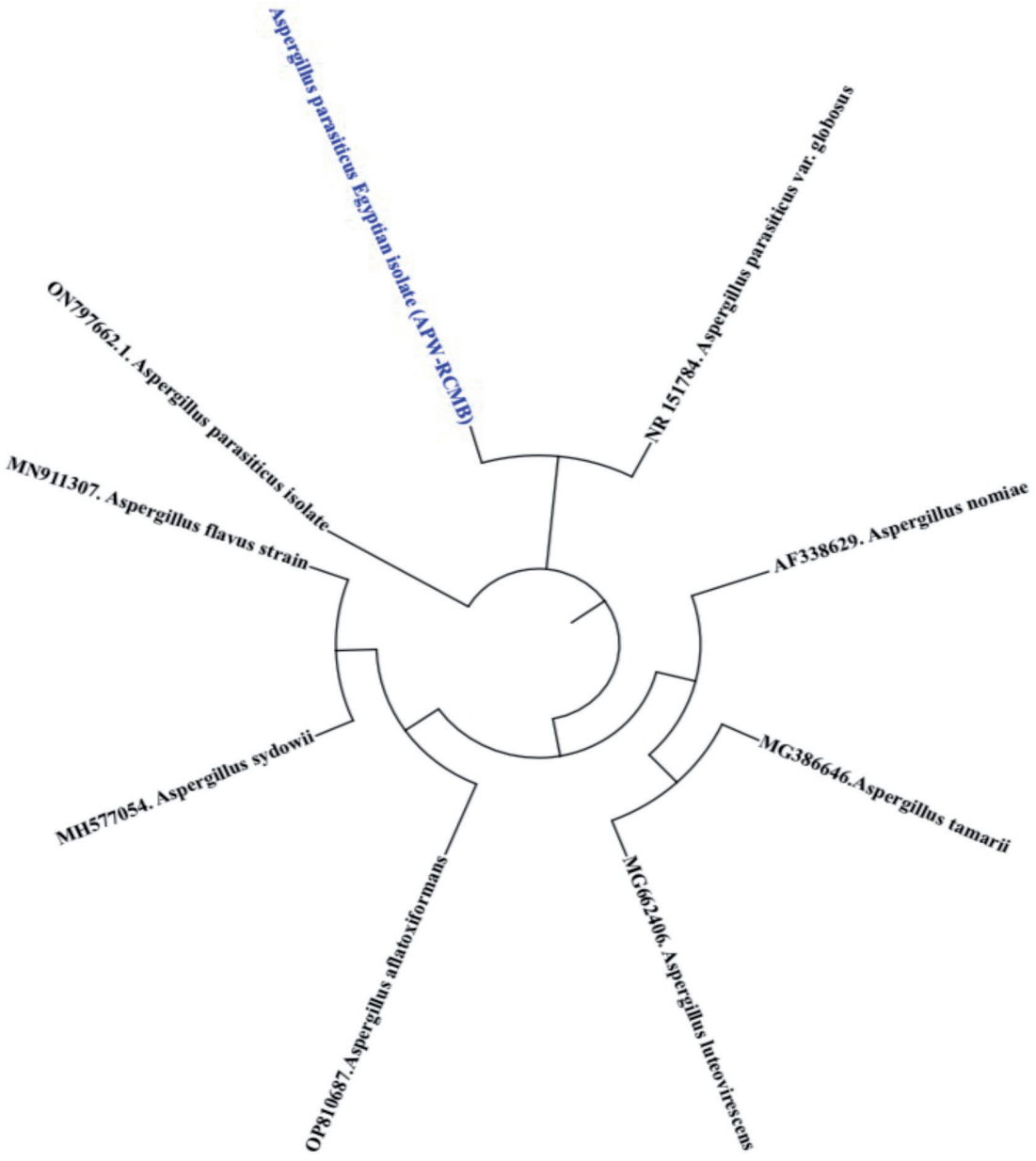

Fig. 2. Phylogenetic tree of *Aspergillus parasiticus* (APW-RCMB) as the most common fungal isolate from all water sources (generated by iTOL software).

Table 3. Dry weights measurements of the filamentous fungal isolates used after treated with different ozone doses for 5 minutes at room temperature (Data are represented as means ±S.D) (*) refer to significant difference between control and this treatment ($p \leq 0.05$).

Ozone Doses / Fungal Strain	Control	10 µg/cc	20 µg/cc	30 µg/cc	40 µg/cc	50 µg/cc	60 µg/cc
Aspergillus parasiticus	2.2±0.1	1.30*±0.1	0.32±0.1	0.09±0.01	0.03±0.01	0.00±0.02	0.00±0.0
Aspergillus terrus	4.2±0.2	3.01±0.2	2.8±0.1	2.2±0.1	1.99*±0.1	1.4±0.1	1.73±0.1
Aspergillus niger	4.0±0.3	3.5±0.1	2.8±0.1	2.50±0.2	1.35*±0.2	0.48±0.2	000±0.01
Penicillium purpurogenum	6.5±0.4	5.10±0.1	4.9±0.1	5.4±0.3	2.1*±0.2	1.9±0.2	1.6±0.1
Alternaria alternate	2.80±0.2	1.60±0.2	0.27*±0.1	0.15±0.2	0.05±0.02	0.01±0.02	0.03±0.01
Ulocladium botrytis	5.92±0.3	5.45±0.2	5.3±0.1	4.5±0.1	3.51±0.2	1.62*±0.2	1.64±0.01

0.00 = NO GROWTH.

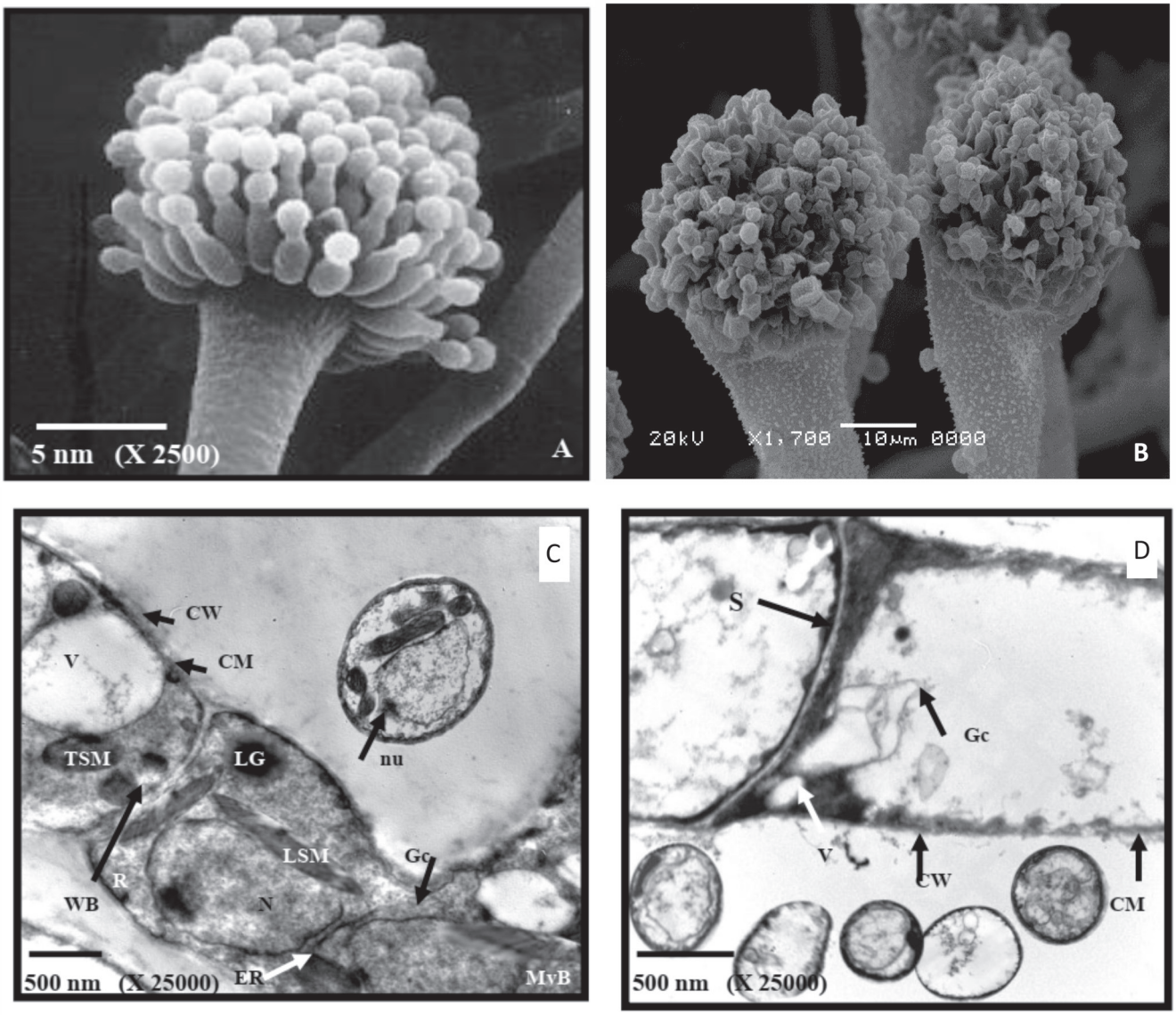

Fig. 3. (A, B) SEM images ; (C, D) TEM images showed cellular alteration in the structure of *Aspergillus parasiticus* upon exposure to10µg/cc ozone for 5 min (CW: Cell wall, CM: Cell membrane, ER: Endoplasmic reticulum, V: Vacuole, N: Nucleus : nu: nucleolus, TS: Transvers section of mitochondria, LSM: longitudinal section of mitochondria, WB: Wide septa, GC: Golgi system MVB: multiple vacuoles, R: ribosome (LG - Left panel : Control ; Right panel : treated). A clear shrinkage of the cellular surface could be seen using SEM as well as disintegration of internal organelles could be seen using TEM.

Table 4. The effect of various doses of ozone and chlorine on sewage and plant exchange water (Data are represented as means where * refer to significant where ($p \leq 0.05$).

		Al-azhar waste water								
Heavy metals	Control	Ozone doses						Chlorine concentration		
		µg/cc 10/5 m	µg/cc 20/ 5 m	µg/cc 30/ 5 m	µg/cc 40/ 5 m	µg/cc 50/ 5 m	µg/cc 60/ 5 m	50 PPM	100 PPM	200 PPM
Fe	8.74	2.54±0.1*	2.44±0.1	2.34±0.1	2.14±0.1	2.04±0.1	2.00±0.1	6.5±0.1	2.1±0.1*	00
Cu	16.14	8.86±0.1*	8.70±0.1	8.60±0.2	8.30±0.3	8.10±0.1	7.90±0.2	12.8±0.2	4.5±0.2*	3.2±0.2
Zn	13.38	00	00	00	00	00	00	11.6±0.1	2.4±0.1*	1.08±0.1
Cd	1.88	00*	00	00	00	00	00	1.2±0.2	00*	00
Hg	9.70	4.0±0.1*	3.8±0.2	3.5±0.3	3.3±0.1	3.2±0.1	3.0±0.1	6.3±0.1	3.8±0.1*	0.95±0.1
		El-Menia water treatment plant								
Heavy metals	Control	Ozone doses						Chlorine concentration		
		µg/cc 10/10m	µg/cc 20/ 20m	µg/cc 10/ 10m	µg/cc 20/ 20m	µg/cc 50/ 5 m	µg/cc 60/ 5 m	50 PPM	100 PPM	200 PPM
Fe	2.64	00*	00	00	00	00	00	1.38±0.1	00*	00
Cu	14.11	11.86±0.2	11.76±0.3	11.56±0.3	11.36±0.3	11.26±0.1	11.16±0.1	8.4±0.2	5.6±0.1*	2.6±0.1
Zn	6.75	00*	00	00	00	00	00	4.2±0.1	2.7±0.1*	0.95±0.1
Cd	4.5	00*	00	00	00	00	00	2.0±0.1	00*	00
Hg	5.4	2.9±0.2*	2.8±0.1	2.6±0.1	2.4±0.2	2.3±0.1	2.2±0.1	3.95±0.1	0.7±0.1*	00

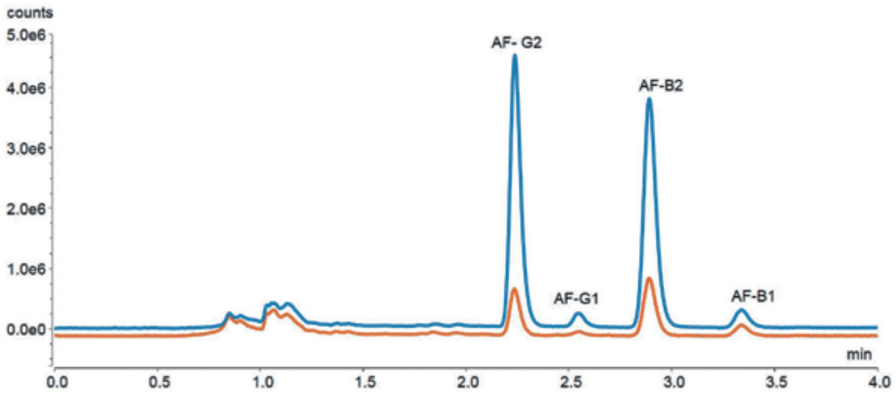

Fig. 4. HPLC graphs separation of aflatoxins secreted by *A. parasiticus* control (blue line) and upon treatment using 10 µg/cc ozone at for 5 minutes (orange line) (a significant reduction ($p \leq 0.05$) in the levels of produced aflatoxins after treatment).

in recent years [30]. The depletion of fungal spores by ozone in water has been documented much less frequently than that of bacteria and viruses, according to prior investigations [31-34]. In the present investigation, six different fungal species were isolated from different water sources, which were morphologically identified, and *A. parasiticus* was the most common fungal species in all examined sources, further identified using the 18SrRNA genetic method and deposited in the gene bank as APW-RCMB with accession number OR511577.1.

The microbial load in drinking water, wastewater, food products like vegetables, fruits, and meat, as well as machinery used for food processing, can be reduced quickly and affordably by the use of ozone (O_3) treatment [35-38]. In the present work, various doses of ozone 10 µg/cc, 20 µg/cc, 30 µg/cc, 40 µg/cc, 50 µg/cc, and 60 µg/cc were applied for five minutes, where 10 µg/cc dramatically reduced the dry weight of *A. parasiticus.* While 40 µg/cc was the most efficient dose for isolated fungi. In accordance with a study done on barley seeds, *Aspergillus* sp. growth was inhibited when ozonated

Table 5. Quantitative analysis of the aflatoxins (µg/ ml) of the fungal control sample and upon treated using 10 µg/cc of ozone for 5 minutes (Data are represented as means ±SD).

RT (minutes)	Compound	Concentration (µg/ ml) in Control sample	Concentration (µg/ml) in Treated sample *
2.3	AFG2	14.07±0.2	4.17±0.1
2.5	AFG1	3.55±0.1	0.75±0.1
2.7	AFB2	10.50±0.2	3.50±0.1
3.4	AFB1	3.51±0.1	1.51±0.1

*A significant reduction in the aflatoxins levels upon treatment using 10 µg/cc of ozone for 5 minutes

water was administered to the seeds [39]. *Penicillium citrinum* and *Aspergillus flavus* colonies were reported to be effectively inhibited from growing normally when exposed directly to gaseous O_3 [40].

O_3's potent oxidizing activity or reactive oxygen species (ROS) produced during the disintegration are thought to be the mechanisms underlying its antifungal capabilities [41]. According to Cho et al. [42], O_3 directly degrades the lipids and glycoproteins found in the fungal cell membranes. O_3 and ROS cause disruptions in the sulfhydryl groups of enzymes that are both apoplastic and intracellular [43]. In live cells, O_3 also compromises the integrity of the nucleic acids [44]. One of the key factors determining a microorganism's susceptibility to sanitizers, including O_3, is the external layer of its cells and the makeup of those cells [45]. In the present study, examination of *A. parasiticus* affected by 10 µg/cc of ozone disintegrated the fungal cells, which were examined using transmission and scanning electron microscopy.

Some heavy metals, like lead and mercury, are hazardous to the health of living microorganisms [46, 47]. Others, like zinc or copper, are crucial micro-elements in the metabolic functions incorporating place in microorganisms when present in low concentrations. Surface waters often do not have levels of heavy metals over the permissible limits. However, there have been instances of considerable water pollution with these substances, for instance from China [48] or Cameroon [49], which may pose serious risks to the ecosystem. Heavy metal levels in surface waters are more likely to be raised in mining regions [50]. The main processes that remove heavy metal ions from water include coagulation, precipitation, and adsorption. It finds out that the type of coagulant applied and the overall conditions of conducting the procedure have an impact on how effectively metals are removed during the coagulation process. A notable reduction in the amount of metal ions in the filtered water was obtained by incorporating ozone oxidation [51]. In the present study, using 10 µg/cc of ozone for 5 minutes decreased the heavy metals levels. In accordance with other studies that suggest using ozone as a coefficient approach for the elimination of heavy metals from industrial effluent [52].

In the present, using 10 mg/cc for 5 minutes reduced toxins production secreted by *A. parasiticus*. The most common mycotoxins are aflatoxins, which are extremely harmful byproducts of several *Aspergillus* species. Aflatoxin contamination has been found in drinking water, wastewater, and surface water, particularly tap and bottled water. Uncertainty surrounds the precise origins of pollution in water. The best potential for adsorption was found with minerals, while the best rates of decomposition were found with gamma and UV irradiations, oxidoreductases, and ozone [53].

Conclusions

Ozone at a dose of 10 µg/cc for 5 minutes could potentially be applied to de-contaminate *A. parasiticus* as a fungal species, which might produce aflatoxins in water through alteration of fungal structure. This dose of ozone could decrease heavy metal levels, thus reducing its harmful impact on human health.

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Conflict of Interest

The author confirms that they have no conflict of interest.

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