

Ultraviolet germicidal irradiation in tap water contaminated by *Aspergillus* spp

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Keywords

Tap water • *Aspergillus* spp • UV disinfection • Water disinfection • Fungi

Summary

We investigated the effect of ultraviolet germicidal irradiation (UVI) from a low-pressure mercury lamp on several pathogenic *Aspergillus* spp. including *A. flavipes*, *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. nidulans*, *A. niger*, *A. terreus*, *A. ustus* and *A. versicolor* suspended in tap water under laboratory-scale conditions. It was shown that within 10 s of exposure, time species such as *A. glaucus*, *A. nidulans* and *A. ustus* were completely inactivated, while 40 s were needed for

the elimination of all the species tested. *A. flavus* and *A. niger* were found to be less susceptible than other species. Based on these results we conclude that UV disinfection could effectively inactivate *Aspergillus* spp. in tap water. Such disinfection could be used to reduce potential exposure of high-risk patients to fungal aerosols, particularly in hospital settings, where point-of-use (POU) UV light devices could be installed to provide safe water at a very low cost.

Introduction

Fungi belonging to the genus *Aspergillus* are ubiquitous and are found in soil, decaying vegetation and various aquatic environments. Several studies have also demonstrated their presence in drinking water and their ability to resist chlorine or other chemical disinfectants [1-7]. Water distribution systems in hospitals could serve as potential indoor reservoirs for microorganisms such as *Aspergillus* species and other molds, mainly through aerosolization of fungal spores. The presence of *Aspergillus* spp. in hospital water distribution systems has been shown in many studies [8-12]. In addition, *Aspergillus* spp. could reside in biofilms in municipal water distribution systems, which might increase their overall concentration in tap water [13-15]. This could represent a significant public health problem since it has been established that several species are the cause of nosocomial aspergillosis, primarily affecting immunocompromised individuals, and are the second cause of nosocomial fungal infections, which are particularly difficult to prevent and treat [16]. Furthermore, although hospitals have adopted preventive measures such as high-efficiency particulate air (HEPA) filters and laminar air flow (LAF), the incidence of aspergillosis continues to rise [17, 18]. This suggests that, in addition to airborne infection, there may be other sources of aspergillus infection in hospitals, including water systems, the so-called *Wet route* of transmission for human systemic aspergillosis [8, 19, 20]. In this regard, it has been shown that the concentration of airborne *Aspergillus* spp. is significantly higher in the areas where the use of water is greater compared to other areas [19].

Ultraviolet germicidal irradiation (UVGI) is a physical technology used for the disinfection of drinking water

thanks to its proven ability to act against many microorganisms, including (oo)cysts of *Cryptosporidium* and *Giardia*, two pathogens of major importance for the safety of drinking water [21, 22].

The UV irradiation technology has several advantages. Firstly, it does not produce undesirable secondary effects often associated with traditional chemical treatments, such as the generation of disinfection byproducts (DBPs), which appear to have mutagenic and/or carcinogenic effects [23]. Second, at the dosage used to treat drinking water (40 mJ cm⁻²), UV irradiation does not significantly change the water's characteristics [24]. Finally, UVGI as a point-of-use sterilizing system can provide safe water at very low cost [25].

To our knowledge, few studies have been conducted on the inactivation of waterborne *Aspergillus* spp. by UV irradiation [26-28]. Furthermore, these investigations report the results of only a few species such as *A. fumigatus*, *A. flavus*, *A. ochraceus* and *A. niger*. Hence, the aim of the present study was to add to the current body of knowledge regarding UV-C irradiation efficiency in inactivating nine *Aspergillus* spp. in tap water.

Materials and methods

FUNGI STRAINS AND TEST INOCULUM CONIDIAL SUSPENSIONS

The following strains of *Aspergillus* spp. were selected for this study and obtained by environmental samples including hospital setting: *A. flavipes*, *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. nidulans*, *A. niger*, *A. terreus*, *A. ustus* and *A. versicolor*. All strains were identified in

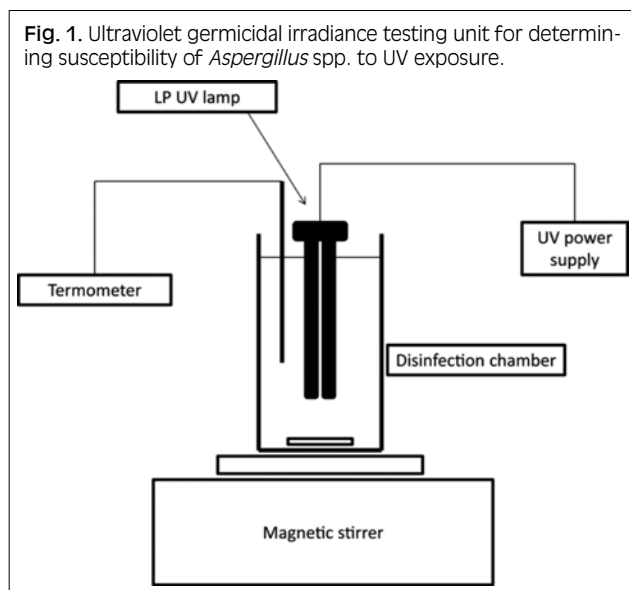
our laboratory using traditional techniques for the isolation and identification of filamentous fungi [29] and subcultured on Potato Dextrose Agar (PDA) at 35°C at least twice to ensure purity and viability. Conidial suspensions for each isolate were prepared according to the CLSI M38-A protocol with some modification [30]: the mould was grown on PDA agar slants for 7 days at 35°C, each slant was flooded with 2 ml of sterile 0.85% NaCl solution containing 0.05% Tween 80 and then gently probed with a pipette tip. The resulting mixture was collected in a sterile tube and the heavy particles were allowed to settle for 3 to 5 min. The upper homogeneous suspensions containing conidia were vortexed for 15 s and the transmittances (530 nm) of the mixture suspensions were adjusted by spectrophotometer to provide a final test inoculum of about 10^6 CFU ml⁻¹. The inoculum titres of each *Aspergillus* spp. were confirmed by plating serial dilutions of the suspensions onto PDA plates.

TAP WATER AND UV SOURCE

Samples of water were taken from a municipal water supply and analyzed to exclude the presence of filamentous fungi using the spread plate technique, a standard method for the examination of water and wastewater [31]. UVT, namely the measure of the fraction of incident light transmitted through water sample, was made in a spectrophotometer at a wavelength of 254 nm. The main characteristics of this water are listed in Table I. UV-C irradiation was performed using a 18 W low pressure mercury lamp (Philips TUV PL-L 18 W 4 pin) with a monochromatic light output of 254 nm providing an average radiation intensity of 3.2 mW cm⁻².

OPERATION OF LABORATORY-SCALE UV EXPOSURE CONDITIONS

Experiments were performed using a UV reactor (Fig. 1), which consists of a cylindrical plastic chamber (250 mm long, 115 mm inner diameter, effective



Tab. I. Typical characteristics of the municipal water supply.

Parameter	Unit	Value
Conductivity	$\mu\text{s cm}^{-1}$	467.18
pH		7.92
Total hardness	mg l ⁻¹	242
Turbidity	ntu	0.28
UVT 254 nm*	%	99
Aluminum	mg l ⁻¹	33.83
Ammonium	mg l ⁻¹	nd
Arsenic	mg l ⁻¹	nd
Calcium	mg l ⁻¹	80.7
Chlorides	mg l ⁻¹	28.89
Chlorine residual	mg l ⁻¹	0.16
Fluorides	mg l ⁻¹	0.25
Iron	mg l ⁻¹	14.81
Magnesium	mg l ⁻¹	16.62
Manganese	mg l ⁻¹	nd
Nitrate as N	mg l ⁻¹	3.7
Nitrite as N	mg l ⁻¹	nd
Phosphate	mg l ⁻¹	nd
Potassium	mg l ⁻¹	0.8
Sodium	mg l ⁻¹	20.33
Sulfate	mg l ⁻¹	57.41

Information from municipal authority; nd: below the limit of detection; * our determination.

volume 2.6 l) containing 2000 ml of water contaminated with 2 ml of each conidial suspension prepared as described above. The lamp was then immersed in the contaminated water. The final strength of the spores in the water was about 10^3 CFU/ml and during the experiment, the sample was maintained at 20 °C under continuous magnetic stirring to ensure the homogeneity of the suspension. The radiation fluence level ranged from 0 to 192 mJ cm⁻² and was calculated as a product of average radiation intensity (mW cm⁻²) and irradiation time (s). At time 0 s (not irradiated control) 10, 20, 40 and 60 s of UV irradiation sample aliquots were collected and tenfold serial dilutions were performed in 0.85% saline solution and 100 μ l of each dilution suspension was spread-plated on PDA media to enumerate CFU/ml after incubation at 30 °C for 3-5 days. Experiments were repeated at least four times for each species.

PHOTO-REACTIVATION AND DARK REPAIR ASSAY

In order to assess the effective ability of complete inhibition of the UV radiation, tests of photo and dark repair were carried out at the exposure times that cause the complete inactivation of each species. Photo and dark repair experiments were assessed by CFU viability assay. Briefly, after the UV fluence, the sample of 10 ml of liquid conidial suspension, at the same concentration as described above, was introduced into 60-by 15-mm culture petri dishes. The sample was maintained with slow stirring at a distance of 50 cm from the fluorescent lamps (Osram 18 W/L20 4000K) and irradiated for 30 min at room temperature (photo-reactivation) in a laminar air flow cabinet. For the dark repair another 10 ml of liquid conidial suspension were taken and introduced into 60-by 15-mm culture Petri

dishes and left to stand for 4 h in darkness at room temperature. The sample was then serially diluted in 0.85% saline solution and 100 μ l of each dilution suspension was spread-plated on PDA media to enumerate CFU/ml after incubation at 30 °C for 3-5 days.

STATISTICAL ANALYSIS

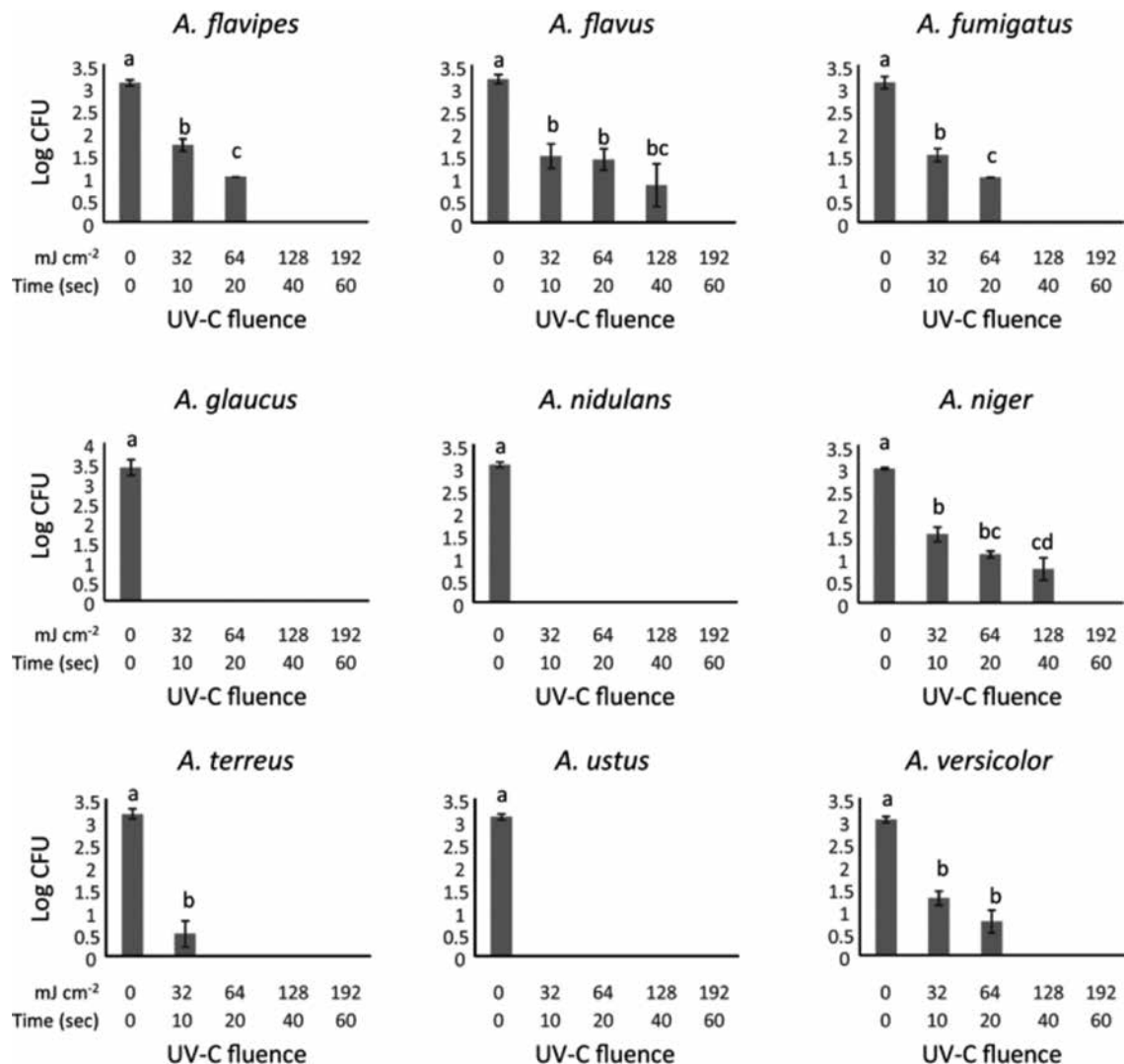
Analysis of variance was performed by one-way ANOVA followed by Tukey's post hoc test. Statistical differences of $P < 0.05$ were considered to be significant.

Results

The susceptibility of the *Aspergillus* spp. to ultraviolet irradiation for various exposure times is shown in Figure 2. Ten seconds of exposure time, equivalent to 2 mJ cm^{-2} of irradiation fluence, is sufficient to cause the complete inactivation of the following species: *A. glaucus*, *A. nidulans* and *A. ustus*, while other species show

reductions of conidial concentrations between 1.38 log and 2.67 log. After 20 s of exposition to UV irradiation (64 mJ cm^{-2}) *A. terreus* is also completely inactivated, whereas the other species such as *A. flavus*, *A. niger*, *A. flavipes*, *A. fumigatus* and *A. versicolor* undergo a reduction of 1.8 log, 1.91 log, 2.09 log, 2.11 log and 2.27 log, respectively. With an exposure time of 40 s (128 mJ cm^{-2}), only *A. flavus* and *A. niger* were still recovered, although viable biomass was significantly reduced (2.23 log and 2.37 log respectively) compared to initial concentrations. After 40 s of exposure time no viable species were found. Regarding DNA repair, we had no evidence of either light or dark repair of DNA damage caused by UV-C irradiation performed using a low pressure mercury lamp under the above mentioned conditions. Values of nephelometric turbidity units (NTU) and UV transmittance (UVT), on average 0.27 and 99% respectively, were always within the limits recommended for the treatment of tap water with UVGI.

Fig. 2. Effect of UV fluence on inactivation of *Aspergillus* spp. Results are means of four independent conidial suspensions \pm SEM for single species; values without common letters are significantly different ($P < 0.05$).



Discussion

Interest in using UV light treatment to disinfect drinking water is growing due to its ability to inactivate pathogenic micro-organisms without forming DBPs and its effectiveness against several pathogens that are resistant to commonly used disinfectants such as chlorine. Many investigations have been made to assess the dose of UV irradiation necessary for effective bacterial disinfection of drinking water [21, 32, 33], while less data are available concerning the effect of UV irradiation on fungi in potable water.

The efficacy of UV irradiation in inactivating fungi in potable water is very important with regard to water distribution systems in hospitals. Such systems are potential indoor reservoirs for these species and other molds, mainly through aerosolization of fungal spores, which could directly affect human health. Thus, we tested the antifungal efficacy of UV irradiation performed with a UV-C low-pressure mercury lamp in tap water to eliminate the spores of 9 species in the *Aspergillus* genera. Using this system, we showed that within 40 s of activity of the UV lamp (128 mJ cm⁻²) it is possible to remove most of the species tested and achieve a significant reduction in the more resistant forms/species such as *A. flavus* and *A. niger*. The latter species are no longer detectable after 40 s of exposure time. In our previous study we found comparable effectiveness of UV radiation for the disinfection of swimming pool thermal water contaminated by dermatophyte fungi [34].

Standards for UV devices in some European countries establish a minimum UV fluence in drinking water of 40 mJ cm⁻², while others recommend doses that should never exceed 140 mJ cm⁻² to avoid significant changes in the physical and chemical characteristics of the treated water [21]. The UV doses employed in this study were within these limits.

Our results confirm that 9 fungi belonging to the *Aspergillus* genera, in particular *A. flavus* and *A. niger*, have a greater resistance to UV irradiation compared to other microorganisms, although the methodology used in our experiments is different from that proposed by other authors who employed continuous flow UV reactor testing or collimated beam testing [22, 26-28, 32, 33, 35, 36]. Furthermore, higher resistance of *A. flavus* and *A. niger* is also described by other authors in tests conducted in water [23], whereas *A. fumigatus* showed the highest resistance in tests conducted in air [37]. The higher UV tolerance of fungal spores belonging to the *Aspergillus* genera, such as *A. niger*, compared to other microorganisms is probably due to their particular cellular structure, which includes the presence of pigments as has been observed in melanized fungi [38]. Water turbidity can protect organisms against radiation and therefore significantly influence the process of UV radiation disinfection [21, 33]. However, in our experiments, both the values of turbidity and UVT were comfortably within the limits recommended for the use of this technology.

The data concerning photo and dark repair experiments confirm the complete inactivation of all fungal species tested within 40 s of UV light exposition.

Conclusions

Present study provides additional information on the effectiveness UV-C radiation in tap water to inactivate potential pathogenic species of fungi belonging to the *Aspergillus* genera. It has been widely shown that these species can survive conventional disinfection treatments, and therefore water distribution systems might be reservoirs for fungi, particularly the *Aspergillus* spp. Hence, in order to eliminate potential exposure to this risk, particularly in hospital settings such as in high-risk wards, we support the suggestion of several authors who call for the installation of point-of-use (POU) UV light devices that are easy to install and provide safe water at very low cost [11, 25]. In such systems working in full-scale UV reactors, the flow device must be adjusted taking into account the data we obtained regarding exposure time to UV radiation necessary to provide complete elimination of fungi.

Moreover future experiments will be conducted for the evaluation of UV-C radiation against the biofilm formation by *Aspergillus* spp. in combination with other filamentous fungi, especially those containing melanin pigments such as dermatophytes.

Acknowledgements

The authors declare no conflict of interest.

Authors' contributions

MS designed the study and performed data analysis and manuscript preparation. GFS and MDS carried out data collection and analysis and performed statistical analysis. GB carried out the technical revision of the manuscript. All authors have critically read and revised the manuscript and approved the final version.

Revision of the test by a English mother-tongue revisor.

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