Effect of ozone gas on cultures of *candida albicans* and *aspergillus fumigatus*: evaluation of two ozonation equipment

Maicon Henrique Caetano
Universidade Estadual Paulista Julio de Mesquita Filho
João Paulo Zen Siqueira
Faculdade de Medicina de Sao Jose do Rio Preto
Adriano Menis Ferreira
Universidade Federal de Mato Grosso do Sul
Denise de Andrade
Universidade de Sao Paulo Escola de Enfermagem de Ribeirao Preto
Alvaro Lopes-Sousa (alvarosousa@usp.br)
Universidade Nova de Lisboa https://orcid.org/0000-0003-2710-2122
Liliane Moretti Carneiro
Universidade Federal de Mato Grosso do Sul
Herica Emília Félix de Carvalho
Universidade de Sao Paulo Escola de Enfermagem de Ribeirao Preto
Marcelo Alessandro Rigotti
Universidade Federal de Mato Grosso do Sul
Margarete Teresa Gottardo de Almeida
Faculdade de Medicina de Sao Jose do Rio Preto

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Abstract

Background

The use of O 3 as antimicrobial has stood out as a useful chemical compound for disinfection processes and/or sterilization due to its high oxidant power. Antimicrobial effects of ozone gas (O 3), produced by two commercial equipment on cultures of two fungi were studied.

Method

Petri dishes were plated with concentrations of Candida albicans and Aspergillus fumigatus and placed on a countertop at three distances, 30 cm, 1 m and 2 m, in three directions of exposure of O 3, with and without air conditioning.

Results

Both equipment showed potential antifungal activity; however, the Mod. I generator ($p<0.05$) presented twice the O 3 flow rate. The results of the inhibitory activity of O 3 of Mod. II, except the cultures of Candida albicans at the distance of 2 m and, of Aspergillus fumigatus , at distances of 1 m and 2 m, showed significant differences ($p<0.05$). Candida albicans was more sensitive to O 3 ($p<0.05$) than the filamentous fungus Aspergillus fumigatus. The experiments conducted with the air conditioning turned off proved the great antimicrobial activity of O 3 . The worst result was observed for the dishes of Aspergillus fumigatus , at the distance of 2 m and near the wall and the best for the central dishes, at the distance of 30 cm, of Candida albicans.

Conclusion

This method of disinfection by O 3 shows a feasible antimicrobial potential to establish new protocols for hygiene and hospital disinfection, reducing environmental contamination by fungi.

1. Background

Fungal infections are of enormous importance in the scenario of nosocomial diseases in Health Care Institutions, with increasing morbidity and mortality rates. Microorganisms transmitted by air, water and/or food can contaminate body surfaces and remain in the hospital environment, favoring the risk of infectious diseases. Several fungi such as opportunists or primary pathogens are frequent agents, especially the species Candida spp. and Aspergillus spp. [1–4].

Candida albicans (C. albicans) is part of the normal microbiota; however, it can cause infections by ruptured biological balance, by predisposing pathological, mechanical, physiological or immunological conditions [5–7]. The Aspergillus fumigatus (A. fumigatus) is a ubiquitous saprophytic fungus that releases millions of conidia into the environment which is a common cause of invasive infections when inhaled, as well as being an allergen agent [8–9].

The use of O_3 as antimicrobial has stood out as a useful chemical compound for disinfection processes and/or sterilization due to its high oxidant power [10]. Although the mechanisms of its action are not fully understood, O_3 is known to act on cell walls given the oxidation of glycopeptides, glycoproteins and amino acids, modifying permeability and causing lysis. O_3 is recombined with cytoplasmic elements when reaches the inside of the cell, causing the oxidation of amino acids and nucleic acids, causing cleavage and consequent cell death. O_3 also causes the collapse of cellular enzymatic activity due to its action in the sulfhydryl compounds of enzymes, in addition to altering the purine and pyrimidine bases of nucleic acids [10–12].

Our study sought to compare the antifungal activity of gas O_3 produced by two commercial equipment, against cultures of C. albicans and A. fumigatus.

2. Methods

2.1. O_3 generators – technical specifications

We used two equipment from OZON® company, called GEO 20000/AR-TD (Mod.I) and GEO 20000/AR (Mod.II), whose specifications are indicated in Table 1.

Table 1
GEO 20000/AR-TD models (Mod.I) and GEO 20000/AR (Mod.II).

Technical specifications	**GEO 20000–AR/TD (Mod.I)**	**GEO 20000/AR (Mod.II)**
Flow rate (m^3/h)/ppm O_3	200–2.0 ppm	100–2.1 ppm
Maximum relative humidity (%)	75	75
Working temperature (°C)	6–35	5–40
Nominal power [MW]	135	127
Power voltage (V)	127	110
Weight (kg)	5.40	4.9
Maximum working area (m^2)		300
Dimensions (cm)	18 × 30 × 47.5	18 × 30 × 37
In which: M^3/H – Cubic meter per hour; % – Percentage; °C – Celsius Degree; W – Watt; V – Volt; Kg – Kilogram; M^2 – Square meter; Cm – Centimeter; PPM – Parts per million.		

2.2. Microbial inoculum and experiment site

Strains of C. albicans (ATCC 90028) and A. fumigatus (environmental origin), belonging to the fungal culture collection of the laboratory of the Department of Dermatological, Infectious and Parasitic Diseases of the Faculdade de Medicina de São José do Rio Preto – FAMERP, São Paulo, Brazil, site where experiments were carried out.

The inoculums of C. albicans and A. fumigatus were prepared and adjusted to the concentrations of microorganisms by spectrophotometry, in correspondence to the 0.5 scale of McFarland (1×10^6 a 5×10^6 cells/mL). Ten Petri dishes containing Brain Heart Infusion Agar (BHI) (Oxoid, Basingstoke, Hants, UK) received 100 µL of the inoculum and were striated with Drigalski spatula. A dish received the same inoculums without being exposed to ozonation, for control.

2.3. O_3 generators – technical specifications Ozonation with generators – Mod.I and II

After sowing, nine dishes were placed on the surface of a granite countertop measuring 2.72 m long and 0.58 m wide, in an experimental room of 9 m^2. The distances of 30 cm, 1 m and 2 m were determined according to the positioning of the O_3 gas generator. The flow targeting of O_3 was evaluated in three different positions, namely A (plates near the wall), B (central plates) and C (plates opposite the wall), as shown in Fig. 1.

Exposure to O_3 gas occurred in a closed environment for one hour. Subsequently, the dishes were closed and incubated at 35 °C. As a control, an inoculated plate was incubated according to the same criteria, without receiving the O_3 treatment. The experiments were carried out in triplicate, including the control.

The procedure described above was conducted at two distinct moments: with air conditioning turned on and off. Temperature and humidity were then recorded.

After 24 h and 48 h of incubation, the dishes cultivating C. albicans and A. fumigatus were observed regarding the number of colony forming unit (CFU/0.1 ml) and compared with the control group.

The data were subjected to statistical analysis, according to arithmetic mean of fungal growth of triplicate tests. Then, the Chi-Squared test (X^2) was applied to verify the associability and dependence between the variables: directions (A, B and C) and distances (30 cm, 1 m and 2 m), with air conditioning turned on and off. When turned on, air conditioning was set to 20 °C and maximum fan power.

3. Results

O_3 generators (Mod. I and II) showed antifungal activity on the two fungal species studied. The dishes showed a reduction in the number of CFU when compared with the control dishes.

The Mod.I device showed greater efficiency in the microbial load reduction process, considering distance and targeting parameters ($p < 0.05$). The results of the Mod.II device showed significant differences ($p <$

0.05), except for the culture of C. albicans at the distance of 2 m (p = 0,3581) and A. fumigatus at distances of 1 m (p = 0,5985) and 2 m (p = 0,4874), proving the associability between the variables.

Considering the distance and direction of the equipment, the dishes at the distance of 2 m in the direction (A) showed the worst antifungal result for A. fumigatus, and the dishes at the distance of 30 cm in direction B showed the best antifungal result for C. albicans, as shown in Figs. 2 and 3.

Table 2 shows the percentage values of inhibition for all variables. The highest inhibition values occurred for the cultures of C. albicans after O_3 activity with both equipment, with significant statistical differences ($p < 0.05$). The inhibition values of A. fumigatus cultures were less expressive, near 50%.

Table 2 Inhibition percentage obtained by the arithmetic mean of the CFU in Petri dishes, at 30 cm, 1 m and 2 m of distances, and directions A, B and C, in the room, with the air conditioning turned on and off, in comparison with the control.

		GEO 2000/AR - TD (Mod. I)					
		Air conditioning off			Air conditioning on		
		30 cm	1 m	2 m	30 cm	1 m	2 m
Candida albicans	A	90	96	93	60	55	42
	B	97	95	94	94	41	38
	C	76	97	90	91	72	31
Aspergillus fumigatus	A	56	56	19	36	24	25
	B	62	72	42	41	25	14
	C	59	35	51	23	29	27
		GEO 2000/AR - TD (Mod. II)					
		Air conditioning off			Air conditioning on		
		30 cm	1 m	2 m	30 cm	1 m	2 m
Candida albicans	A	96	95	96	96	96	94
	B	97	96	96	97	96	95
	C	96	95	95	96	95	94
Aspergillus fumigatus	A	57	57	46	47	50	47
	B	65	49	43	57	50	48
	C	57	48	50	46	48	44

Antifungal activity of O_3 was better for the tests performed in the room with air conditioning turned off in both fungal species. The 83% and 78% inhibition percentages, respectively, of the equipment Mod.I and Mod.II stand out. The values were lower when the air conditioning was on, 28% and 67%, respectively.

Considering the experimental environment, with air conditioning turned on and off, the average temperature recorded were 21 °C and 25 °C and average humidity were 58% and 53%, respectively, with the air conditioning turned on and off.

4. Discussion

Our study is a pioneer in the evaluation of the antifungal action of O_3 gas on the surface of culture medium contaminated by C. albicans and A. fumigatus due to: (1) the test of different distances and places using two equipment to generate this gas; (2) the assessment of air flow interference on antifungal effects.

According to the data, despite observing CFU measures, after ozonation, showed lower number than those of the control group for both experiments, considering all variables: microorganisms, distances, directions, O_3 gas generator and air conditioning turned on or off.

The Mod.I device statistically showed greater efficiency in the microbial load reduction process, regardless of the fungal species tested, which is explained by the different flow of the devices. Although O_3 concentrations in part per million (ppm) are virtually equal, the Mod.I O_3 generator has twice as much flow when compared with Mod.II. Face to an exception of the reduction of CFU with Mod.II was observed, which had no difference in the results with statistical significance of the cultures of C. albicans at 2 m of distance, and A. fumigatus, at 1 and 2 m of distance, regardless of the place of the dishes (A, B and C). An associability or dependence between the directions and distances is proven regarding the other groups, interfering in the antifungal activity of O_3 gas.

In this context, the best experimental condition occurred to C. albicans, at 30 cm of distance, direction B (central), while A. fumigatus, at 2 m of distance and direction A (dishes near the wall), represented the less favorable condition. This may be related to the gas flow rates. In laminar flows, particles move orderly, always maintaining the same relative position. In the turbulent regime, these particles move randomly and irregularly [13], that is, the flow of O_3 gas when reaches the wall causes disorder, thus decreasing antifungal activity when compared with the dishes near the wall, which may have interfered with the O_3 flow, thus justifying the variation in the number of CFU in all distances and directions.

The best antifungal activity in the ozonation process for both devices when the air conditioner was turned off, for both fungal species, may have been observed due to the lack of interference with the O_3 flow, resulting in surfaces more affected by O_3 gas. Unlike when the air conditioning was turned on, with the function of capturing air and filter before throwing it again into the environment [14], the flow of O_3 gas varies when reaching the surface, thus decreasing its antifungal activity.

According to the literature, temperature, relative humidity variation and treatment time lead to a modified O_3 antimicrobial effect [15]. In our study, these factors were maintained equal in all experimental conditions, minimizing possible biases. According to the values we detected, the cultures of C. albicans were more sensitive than A. fumigatus, which corroborates the literature [15]. In fact, A. fumigatus has can grow and survive humidity environments and extreme temperatures, with dispersion of conidia [9]. According to Santana et al [7], C. albicans is less adapted to the diversity of environments outside the human body than A. fumigatus.

Several studies have been conducted aiming at investigating the antimicrobial activity of O_3 gas against fungi, sensitive or resistant, with different exposure time and concentrations. Variables were analyzed for yeast inhibition, germination tube formation and biofilm, proving that O_3 gas in well-established protocols for exposure time always shows excellent antimicrobial activity [5, 12, 17, 18]. Thus, it is evident that our study corroborates other studies in the literature.

Zotti et al. [19] observed phenotypic changes in the colony of A. flavus and A. niger after exposure to O_3 gas, with sharp decrease in growth of both fungi, and change in their natural pigmentation. In addition to the antimicrobial activity, O_3 gas can be expected to inhibit pigments and protein synthesis, with future impairment to virulence factors of pathogenic fungal species.

Surface cleaning is crucial for the control of Healthcare-Associated Infections (HAI) [20]. Unfortunately, health services surface cleaning and disinfection is often overlooked. Cleaning and disinfection practices of the environment, equipment, and surfaces should be implemented and discussed by the Hospital Infection Commissions, together with nursing and cleaning services, developing activities related to environmental hygiene protocols, supervision, and training [4, 21, 22, 23, 24].

Finally, it is important the search for new products, methods, and practices for surface disinfection, and the O_3 gas appears as a promising compound.

5. Conclusion

Our study proved the antifungal potential of O_3 produced by two different devices, according to the described criteria. This is a satisfactory procedure for the decontamination practices due to its fast and easy execution. This procedure provides a new perspective for disinfection of surfaces aiming at a better control of the dispersion of microorganisms. The incorporation of this procedure into hospital hygiene and disinfection protocols may decrease contamination rates and, consequently, fungal infections in health facilities.

The action of O_3 on fungal control is important and the realization of more studies seeking to optimize the best performance of this gas against this group of microorganisms is relevant.

Abbreviations

FAMERP - Faculdade de Medicina de São José do Rio Preto –

CFU - Colony forming unit

CT - Control group

Declarations

Ethics approval and consent to participate

The present study met national and international ethical precepts. The research did not involve humans, and for this reason received ethical waivers by the Human Research Ethics Committee.

Consent to publish

Not applicable.

Availability of data and materials

All data and materials are available upon request to the corresponding author (Alvaro Francisco Lopes Sousa).

Competing Interests

One of the authors of the manuscript (Alvaro Francisco Lopes Sousa) is on the editorial board of BMC Infectious Diseases, as Associate Editor.

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Authors Contributions

MHC, AMF and MTGA collaborated to the conception and design work, material acquisition, analysis, and interpretation of the data that led to the manuscript. JPZS, LMC and MAR contributed to analysis, and interpretation of the data, as well as to the writing of the article. AFLS, DA, and HEFC collaborated to the critical analysis of the content and interpretation of the data in the successive revisions that originated the manuscript. All authors have read and approved the final version of the article.

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Figures

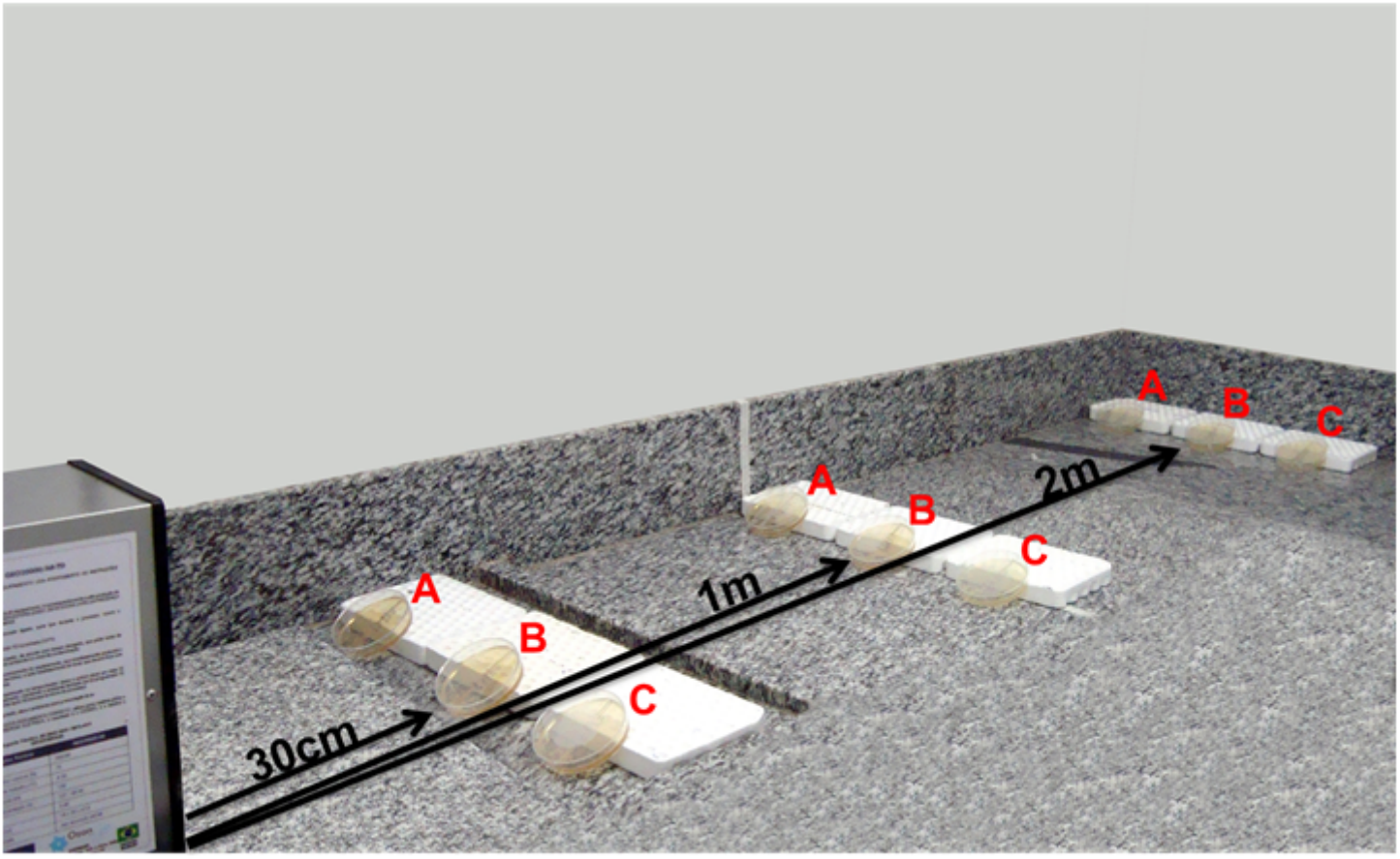

Figure 1

Illustrative photographic image of the direction and distance of O3 generator in relation to the Petri dishes.

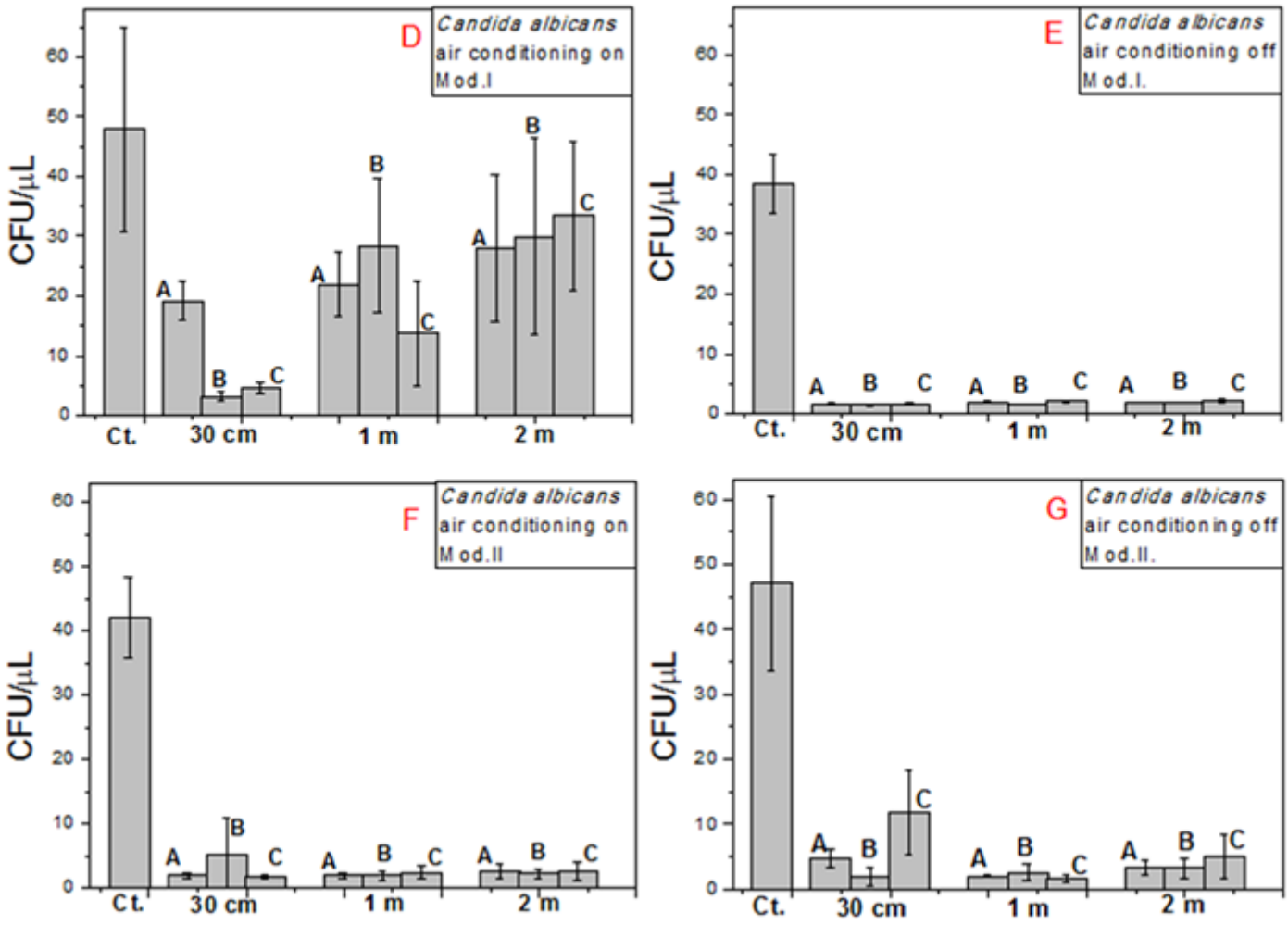

Figure 2

Arithmetic mean and standard deviations of the CFU of C. albicans in the Petri dishes, at 30 cm, 1 m and 2 m of distance and directions A, B and C (related with figure 1), D) air conditioning on, Mod.I, E) air conditioning off, Mod.I, F) air conditioning on, Mod.II, G) air conditioning off, Mod.II. Ct.= Control group.

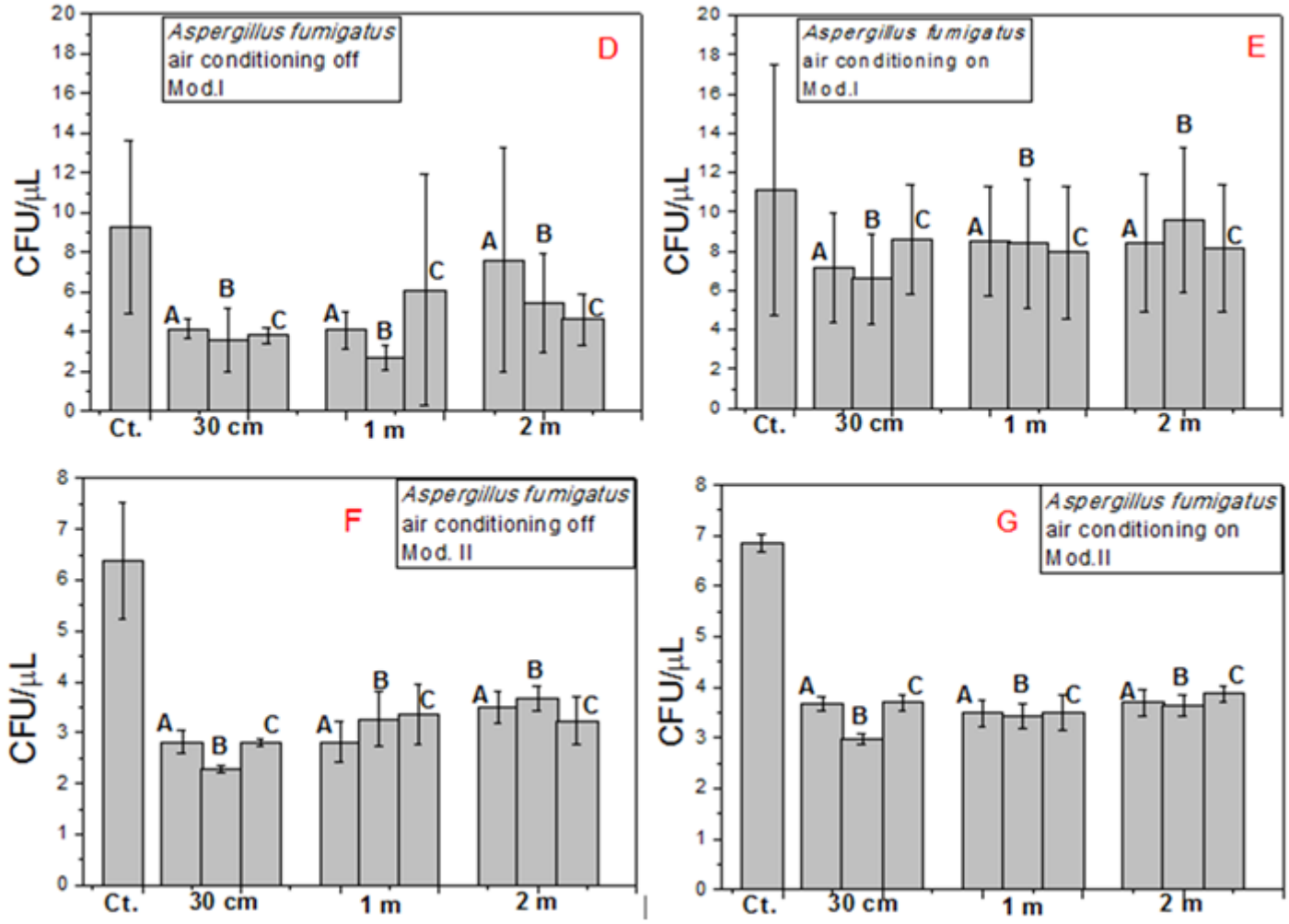

Figure 3

Arithmetic mean and standard deviations of the CFU of A. fumigatus in the Petri dishes, at 30 cm, 1 m and 2 m of distance and directions A, B and C (related with figure 1), D) air conditioning off, Mod.I, E) air conditioning on, Mod.I, F) air conditioning off, Mod.II, G) air conditioning on, Mod.II. Ct.= Control group.