



Effect of the oscillating magnetic field on airborne fungal

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Abstract

This study shows that some species of fungi are affected by the magnetic field, which should be taken into account in studies of airborne fungal and air quality. The aim of this paper was to evaluate the effect of the oscillating magnetic field (OMF) on the behavior of colonies of three fungi genus growth in different culture mediums. The stains were: *Aspergillus niger*, *Cladosporium cladosporioides* and *Penicillium citrinum* and were inoculated in 90 mm Petri dishes with: Malt Extract Agar (MEA), Sabouraud Dextrose Agar (SDA) and Czapek-Dox Agar (CDA). Was applied them OMF of 60 Hz/220 V between 1 and 5 mT during 2 h and then they were incubated 7 days to 28 °C. Colonies size (mm) every day was measured. Stimulation in the colonies size of all experimental conditions was showed; the greatest size of *A. niger* in MEA was notorious. It was demonstrated by statist analyze that only colonies size with 1 mT was significance respect to the control. The effect of OMF on the cellular metabolism was evidenced, as well as: less exudation and major pigmentation of *P. citrinum* in MEA; variation of pigmentation of *A. niger* and *C. cladosporioides* in CDA and increase of conidiogenesis of *A. niger* in SDA. Was concluded that the applied OMF had a major influence on size colony and mycelia pigmentation of *A. niger* that *C. cladosporioides* and *P. citrinum*, independently of the nutritional state according to the culture medium employed in this study.

Keywords Airborne fungal · Magnetic field · Air quality · Fungal physiology

Introduction

Modern indoor environments are designed according to the needs and comfort of many people, but in the construction of buildings of different biological, physical and chemical agents that cause environmental pollution (Yang and

Heinsohn 2007) should be evaluated. For example, as independent of each other international regulations, in a facility should be studied exposure of human beings to the density values oscillating magnetic field (OMF) 50 or 60 Hz and filamentous fungi that form its air mycobiota, since both separate factors can cause damages to health.

The growth of filamentous fungi is due to its apical elongation at their tips accumulation of vesicles called Spitzenkörper (Spk), whose position determines the direction and growth speed of the hyphae and its ramifications (Steinberg 2007). These variables depend on the consumption or depletion of nutrients (Knudsen et al. 2006). In this regard, it is known that the effect of ionizing radiation on the filamentous fungus is related to their nutritional status and there is no genetic effects such as retardation or stimulation of growth and enzyme activity, which can be influenced by this radiation (Casadesus et al. 1985). It is suggested that ionizing and non-ionizing radiation (ex. gamma and UV rays, respectively) kill microorganisms depending on its wavelength, intensity and duration (Tortora and Funke 2007; Rojas-Triviño 2011; Guerrero-Beltrán and Haro-Maza 2013).

Also the presence of substances as sunscreens can scatter or reflect sun radiation (sun screens), among which include

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zinc oxide (ZnO) and titanium dioxide (TiO₂), that solar radiation reduced germination by 42% and 43% for the conidia with and without sunscreens, respectively, while UV radiation does not affect the germination of conidia of *Paecilomyces sp.* without sunscreens (Erika Paola Grijalba et al. 2009). This result is similar to that observed by (Blanco 2013) when applying both radiations on the fungus *Penicillium spp* in mesino limons.

It is argued that different fungi employ defense mechanisms such as the secretion of absorption pigments (Erika Paola Grijalba et al. 2009) by synthesizing melanin to protect against these radiations (Mosso et al. 2002) and there are ultraviolet radiation tolerant strains by repair of DNA damage. However, in some fungi melanization is associated with pathogenicity as in *Aspergillus fumigatus* and the yeast *Cryptococcus neoformans* (Urán et al. 2008). Recently it was discovered that *C. neoformans* increased its growth rate and melanin production when exposed to values of ionizing radiation Ce₁₃₇ 500 times higher than those found in the Thermonuclear Chernobyl and the surrounding area of where this pathogenic yeast was isolated (Dadachova et al. 2007; Dadachova and Casadevall 2008). The electromagnetic exposure has definitely influenced the biosynthesis of catalase and peroxidase enzymes involved in the cellulolytic fungi development. The most perturbing action was exerted by the pulsatory electromagnetic field upon the peroxidase dynamics. This experimental evidence may represent the basis of putative new biotechnological tools in monitoring cellulolytic fungi for industrial purposes (Manoliu et al. 2006).

However, the OMF to 50 or 60 Hz (alternating current) is classified as ionizing radiation and their influence on fungi airborne of the indoor environments is unknown. Thus, there are no regulations that value the possible synergistic interaction between the two factors today. Also, the influence of the magnetic field emitted by electronics appliances in indoor environments on airborne fungi must be taken into account in studies of air quality, environment and health. Therefore, the aim of this study was to evaluate the effect of the oscillating magnetic field (OMF) on the behaviour of the three fungal genera colonies grown in different culture medium.

Materials and methods

Experimental design

The optimal design experiment with Design Expert statistical software version 8.0.6.1 was realized. The following factors were taken into account: OMF density 60 Hz/220 V, incubation time, fungal species and culture medium. Table 1 shows the levels for each factor, obtaining 32 experimental runs which were made at random. The response variable was the colony diameter measured in mm in each time period indicating the statistical program (Mier and Toriello 2002).

The exhibition time was 2 h, according to a previous study, the maximum results were obtained at that time (Anaya et al. 2015). OMF radiation was applied immediately after inoculation to assess germination, because (Willocquet et al. 1996) reported that it was most affected by exposures to simulate sunlight that was applied just after inoculation of the fungus *Uncinula necator*.

Three different culture medium (BIOCEN, Cuba) were used to assess the effect of the OMF based on the nutritional status of the three fungal strains, which were plated in triplicate in 90 mm Petri dishes and incubated at 28 °C for 2–7 days.

To apply OMF solenoid coil was used with air core, with current 60 Hz/220 V through a copper wire of 2 mm diameter wound on a cardboard of 30 cm diameter and 29 cm for resistance of 6.1 Ω. Said coil was characterized by the National Center for Applied Electromagnetism (NCAE) of Santiago de Cuba, Cuba, which generates homogeneous values between 1 and 20 mT magnetic field.

Study strains

The fungal strains used (*Aspergillus niger* Tiegh., *Penicillium citrinum* Thom. and *Cladosporium cladosporioides* Fresen.) were colonies of the most common genera obtained in air quality indoor studies in premises in Havana, Cuba (Anaya et al. 2016, 2019). They were selected considering the growth rate, pigmentation and melanin production.

Table 1 Levels of experimental design factors

Level*	Factors			
	Density of OMF (mT)	Time of incubation (d)	Fungal specie	Culture medium of agar
1	0 (low)	2 (low)	<i>A. niger</i>	Malta Extract
2	5 (high)	7 (high)	<i>P. citrinum</i>	Sabouraud Dextrose
3	–	–	<i>C. cladosporioides</i>	Czapek Dox

*Each of the categories, values or specific forms of the factor

Statistic analysis

The data obtained was analyzed with the statistical program Statgraphics Centurion XV dual classification using ANOVA and multiple range method for the least squares difference (LSD) of Fisher.

Results and discussion

The increased temperature is an important factor in the microbial metabolism, indicating that this phenomenon might affect on cell growth. In this regard, studies with *A. niger* was observed that upon reaching 34 °C during incubation and a difference of 11 °C above the allowable temperature (23 °C) disappeared Spk, and after some time he appeared a new Spk, where born two apical branches, i.e., continued to grow (Reynaga-Pena et al. 1997). On the other hand, the variation of 6 °C (28–34 °C) for 6 h, not caused damages on the growth of the yeast *S. cerevisiae* treated with high frequency OMF (Gos et al. 1997).

In this study, inside the coil maximum temperature of 32.6 °C it was reached, while around him the maximum temperature was 31.2 °C (room temperature) for a difference of 1.4 °C. Therefore, it can be concluded that the effects observed in this study are due to OMF applied.

The variation of the diameter of the colonies of the three fungal strains: was slower and colonies smaller diameter CDA and the strain grew rapidly and reached higher diameter was *A. niger*. The diameter of the control colonies were ordered in decreasing order according to the fungal species *A. niger*, *P. citrinum* and *C. cladosporioides* and according to the culture medium: SDA, MEA and CDA. That is, that the larger diameter of the colony was observed in *A. niger* in SDA and the lowest was obtained in *C. cladosporioides* growing in CDA.

Heeding the effect of the implementation of OMF, no change in the above order of the diameter according to the culture medium. However, the behavior of the fungal colonies was not similar in respect to the controls treated strains for metabolic stimulation (colony diameter variation, exudation, pigmentation and conidiogenesis) was observed. The most statistically significant stimulating effect ($p \leq 0.05$) on the increase in diameter was observed with 1 mT followed by 2 mT and 5 mT was not significant compared to the control.

Research studies of (Ulevičius et al. 2008) demonstrated that the fungal propagules of *Aspergillus niger*, *Paecilomyces puntonii* and *Penicillium expansum* responded to UV radiation distinctively. The propagules of *P. puntonii* were injured without possibility to repair. On the contrary, *P. expansum* propagules repaired after a long enough exposure to UV radiation, but this ability was limited.

Table 2 shows the mean results for each fungal strain in each experimental condition. There were statistically significant differences in the factors studied compared to controls ($p \leq 0.05$), which had positive regression coefficients indicate an increased diameter of the colonies treated with OMF (Table 3).

It should be noted the significance of the interaction between the density of the OMF and culture medium (AD) factors as this indicates that the magnitude of the effect of the OMF applied depends on the nutritional status of the fungus. Being the only quadratic term (B^2) significant model: in this sense, the fungal species (*C*) receiving the magnetic treatment and the time measurement colony diameter (*B* incubation time) is performed is critical and given the highest values of the regression coefficients of the terms CD and BC (12.94 and 6.20, respectively).

The foregoing shows that there is a time when the maximum diameter of the colony stimulated OMF effect also could enhance their metabolism is observed as the availability of nutrients. Data obtained results fit a quadratic model ($y = ax^2 + bx + c$) that predicts 97% of the behavior of the variable ($R^2 = 0.98$), according to the equation $Dc = X_0 \cdot B^2 + X_1 \cdot B + X_2 \cdot A + X_3$, where *Dc* = diameter of fungal colony; X_0 , X_1 and X_2 : regression coefficients; X_3 : constant model; *A*: density of oscillating magnetic field; *B*: incubation time of the fungal strain.

Figure 1 shows the prediction of the increased diameter of the colonies, which was obtained with the mathematical model discussed above. The strains controls the diameter was generally higher in SDA, however, treated for higher BMC values strains the diameter of the colonies was higher in MEA (see scale graphics), although it was more *C. cladosporioides* significant CDA (see steeper the graph from 0 to 5 mT). This being the culture medium fewer nutrients, this result may be related to melanin production, since during the study increased pigmentation was observed with 2–1 mT (Fig. 2a respect to b). However, with SDA the diameter of the colony of *P. citrinum* showed a slight increase to values between 2 and 3 mT. Because the prediction model was quadratic, can conclude that 2 mT is the critical density value of OMF 60 Hz/220 V for several magnetobiological effects on filamentous fungi are observed.

In Fig. 2, the most relevant results of the effect of applied OMF varying the diameter of the fungal colonies, exudation and excretion of pigments can be observed. Figure 2a–d demonstrates a variation in the pigmentation of *A. niger* strains and *C. cladosporioides*. Figure 2e shows the exudation on the control *P. citrinum* colony that was not observed in the treated colonies, which had a green pigment more intense (Fig. 2f–h). In the case of *P. citrinum* strain, increased sporulation corresponds to that reported by (Blanco 2013) with *Penicillium spp* higher concentration of spores of the fungus irradiated with UV light.

Table 2 Results of the matrix experimental design

Run	Density of OMF (mT)	Time of incubation (d)	Fungal specie	Culture medium	Diameter of colony (mm)
1	2	2	<i>A. niger</i>	Malta Extract	45
2	5	5		Agar	68
3	0	7			49
4	5	2	<i>P. citrinum</i>		22
5	0	5			18
6	0	5			17
7	5	7			30
8	0	2	<i>C. cladosporioides</i>		18
9	3	4			32
10	3	7			34
11	5	2	<i>A. niger</i>	Sabouraud Dextrose	45
12	0	4		Agar	83
13	4	7			90
14	4	7			90
15	0	2	<i>P. citrinum</i>		11
16	3	2			18
17	5	4			40
18	0	7			28
19	2	2	<i>C. cladosporioides</i>		8
20	2	2			9
21	5	7			24
22	0	2	<i>A. niger</i>	Czapek-Dox	8
23	4	3		Agar	22
24	1	6			58
25	5	7			40
26	5	2	<i>P. citrinum</i>		10
27	3	6			25
28	3	6			28
29	0	7			15
30	5	2	<i>C. cladosporioides</i>		4
31	0	7			20
32	0	7			18

The control samples not treated with chromium (0 mT). Average for $n = 3$

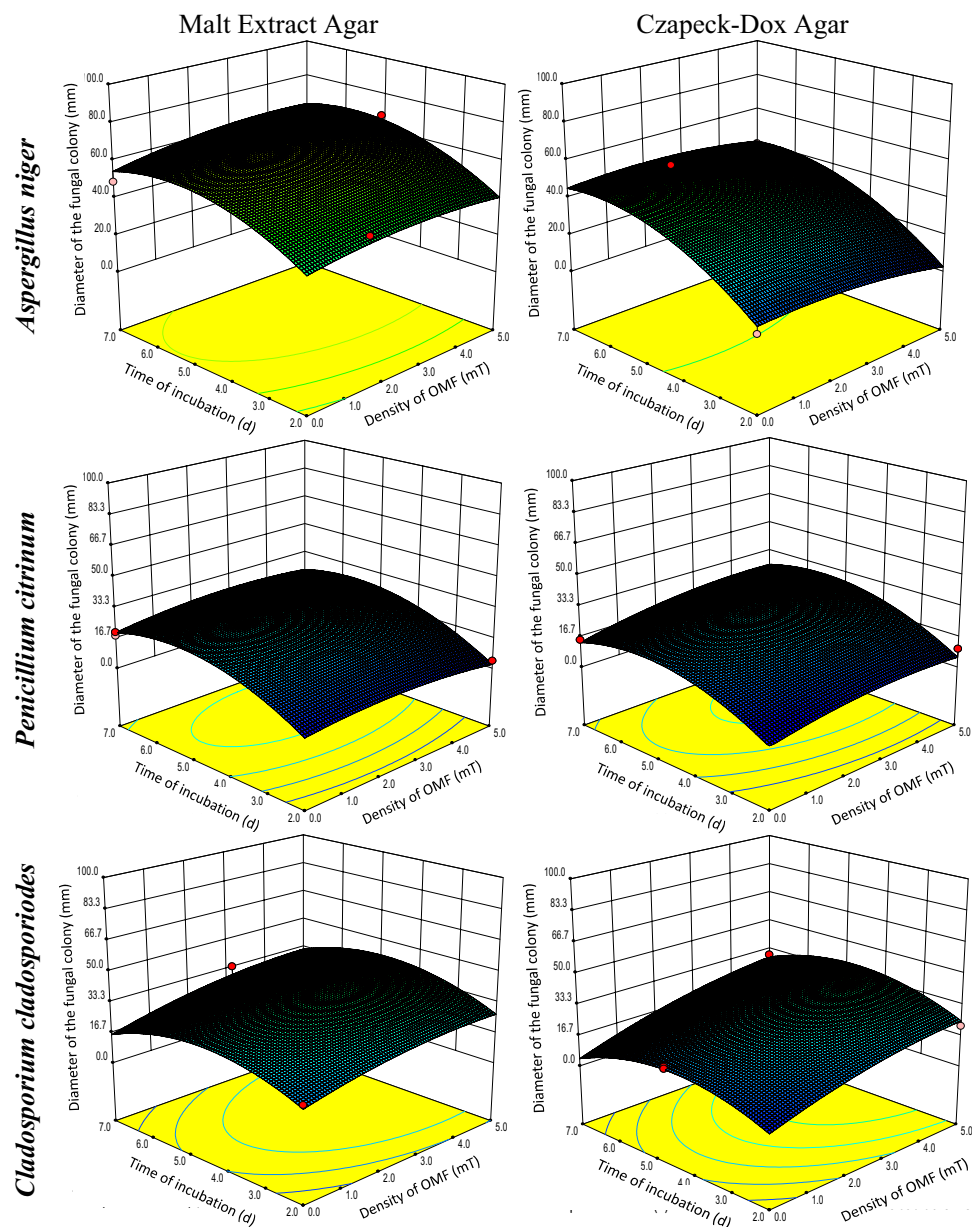
Table 3 Statistical summary of ANOVA of the experimental design ($p \leq 0.05$)

Codificated factor	Regression coefficients	p value
Intercept	42.88	–
A: OMF (mT)	2.36	0.0456
B: Time of incubation (d)	9.51	<0.0001
C: Fungal specie	20.94	<0.0001
D: Culture medium	8.13	<0.0001
AD	4.7	0.0547
BC	6.20	0.0450
BD	3.06	0.0530
CD	12.94	0.0013
B ²	– 12.03	0.0016

These effects of the OMF on growth cellular metabolism and function of nutrients, consistent with those observed in a similar study with the pathogenic bacterium *Streptococcus pyogenes* at 24 °C. OMF was applied 50 Hz in the range of 50–500 mT for 15 h in phosphate buffer solution (saline) and brain–heart–broth (BHB) (nutrient-rich culture medium). It was observed that 300 mT in buffered solution caused an increase in cell metabolism compared to the control, while growth slowed in BHB in the full range of employee OMF (Morrow et al. 2007).

This shows that the OMF applied affects the pigmentation of the colonies, regardless of nutritional status of the fungus, which is showing a similar behavior when applied ionizing radiation on *C. neoformans* (Dadachova et al. 2007) and simulated sunlight radiation of 320 nm on *P. puntonii*

Fig. 1 Predictive response surfaces varying the diameter of the fungal colonies for 7 days, after treated for 2 h with oscillant magnetic field (OMF) of 60 Hz/200 V. The colors in hues from blue to green in the matrix and surface contour lines represent a scale of 4–70 mm in diameter; red and pink dots represent experimental values above and below the values predicted by the quadratic mathematical model obtained by the experimental design



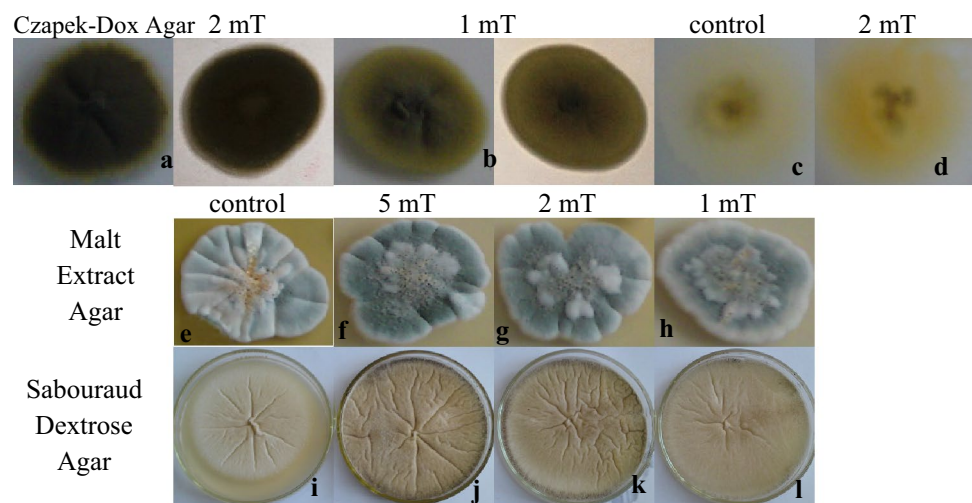
(Ulevičius et al. 2008), higher than 400 nm on *Pythium* sp. (Hughes et al. 2003) or higher than 750 nm on *Alternaria solani* (Stevenson 1988). Therefore, it can be inferred that the magnetobiological effects of alternating current 50 or 60 Hz on filamentous fungi and bacteria are higher the lower the availability of nutrients. This could be explained as an adaptive response to stress (little food and oscillations at the frequency of OMF), which activates metabolic mechanisms to achieve maximum reproduction and survival.

In this regard, Fig. 2i–l shows the increase of conidogenesis of *A. niger* on SDA, coinciding with the maximum size of the diameter of said colony explained above (Fig. 2j). This result agrees with that reported in a similar study in which the static magnetic field (0 Hz) of 0.1 to

3.5 mT stimulation was applied and 10 were observed in 70% germination of conidia of *Curvularia inaequalis* and *Alternaria* (Pál 2006).

This might indicate an adaptation of the genus *Aspergillus* to any radiation, because it was observed that the stressed *A. niger* propagules were recovered after the 80 min exposure to UV radiation and the relative recovery respect with *P. puntonii* and *P. expansum* (Ulevičius et al. 2008). That was similar to the slight resistance of *A. parasiticus* on irradiated chestnuts with 0.25–3.0 kGy of gamma radiation (Cabral et al. 2011). In that sense, several mycotoxins (aflatoxins B₁, B₂, G₁ and G₂, ochratoxin and zearalenone) showed different sensitivity to radiation between 0.9 and 2.5 kGy, no significant decrease for aflatoxin B₁ and B₂ (Cabral et al. 2013).

Fig. 2 Aspect of the fungal colonies after 7 incubation days at 28 °C by effect of exposure to density (mT) of oscillating magnetic field (OMF) at the inoculation time on different culture medium. *C. cladosporioides* (a, b) (reverse and anverse); *A. niger* (c, d); *P. citrinum* (e–h) and *A. niger* (j–l)



Two microwave power densities (10 and 60 mW/cm²) and three times of exposure (5, 30, and 60 min) The results showed that the viability of *Aspergillus versicolor* and *Penicillium brevicompactum* depending on strains, growth conditions, power density of microwave radiation, time of exposure, and varied according to the applied combination of the two latter elements (Górny et al. 2007). So, the magnetic field influences the physiology of airborne fungi. Factor to be taken into account in studies related to indoor air quality and the health of immuno-incompetent people exposed to potentially pathogenic fungi.

Conclusion

It is concluded that the OMF of 60 Hz/220 V and low density values (1–5 mT) affects the cellular metabolism of the filament fungi, causing variation in the exudation, pigmentation and conidiogenesis of them according to their nutritional status. It has a greater influence on the genus *Aspergillus* sp than on *Cladosporium* sp and *Penicillium* sp, and on the size of the colony and mycelium pigmentation, regardless of the amount of nutrients according to the culture medium used.

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