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Brief article

RADIAL GROWTH OF *ASPERGILLUS* AND *PENICILLIUM* SPORES EXPOSED TO LABORATORY DISINFECTANTS

F. I. Okungbowa* and A. O. Usifo

Department of Plant Biology and Biotechnology, University of Benin, PMB 1154, Benin City, Nigeria.

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Aspergillus and *Penicillium* are ubiquitous fungi and are common laboratory contaminants. They have also been isolated from currency notes[2], food and other agricultural products[5], beddings[7], and they constitute a high proportion of the mycoflora of the air[3]. *Aspergillus fumigatus* is known for its implication in human infection of the respiratory tract and production of harmful toxins[6]. In the laboratory, some disinfectants, such as alcohol and sodium hypochlorite, are used to clean work benches and to surface-sterilize. Despite efforts at suppressing/control of these fungi, more often than not they still constitute a menace as they germinate and rapidly colonize the growth media. In this report, the effect of three commonly used disinfectants on radial growth of *A. fumigatus* and *Penicillium* sp. was investigated so as to determine the most effective and the appropriate concentration.

Aspergillus fumigatus and *Penicillium* sp. were isolated from retail “ogbolo” [*Irvingia gabonensis* (Aubry-Lecomte ex O’Rorke) Baill.] seeds purchased from a local market in Benin City. The seeds were teased from apparently healthy portions and cut into about 3 x 1 mm pieces. The pieces were surface-sterilized in 70% ethanol for 3 min, and then rinsed in several changes of sterile distilled water. Thereafter, fresh Sabouraud Dextrose Agar (SDA) plates (containing chloramphenicol 50 µg/ml) were inoculated with five pieces each and incubated at room temperature (28 ± 2 C) for five days. *A. fumigatus* and *Penicillium* sp. were isolated from the SDA cultures and identified by comparing and matching cultural, pictorial and microscopic characteristics with earlier descriptions[1]. Additional identification of *A. fumigatus* was done by microscopic examination[4], but without growing in the recommended medium. Also, since it was not established if the *A. fumigatus* was one of the new species (“absolute” *A.*

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* Author for correspondence; e-mail: fiokun2002@yahoo.com , phone: 234-8055376204.

fumigatus), it was simply identified as belonging to the *A. fumigatus* "aggregate". The two fungi were stored in SDA slants in McCartney bottles at 4 C for future use. The disinfectants used were 70% and 90% methanol, 70% and 90% ethanol, and JIK® (3.85% w/v sodium hypochlorite, Reckitt Benckiser, Nigeria). The sodium hypochlorite was diluted 1:4 v/v. A loop-full (2 mm diameter) of spores of a 4-day old revived culture of each fungus was suspended in 1 ml of appropriate disinfectant and left for 3, 5 or 10 min. Three independent experiments were set up for the three time points, with an interval of 6 h between them for convenience. At day 0, 0.5 ml of the spore suspension of each treatment was transferred to the centre of a fresh SDA plate (fortified with chloramphenicol, 50 µg/ml) and incubated at 28 C. Radial growth of fungus was measured daily. Culture plates inoculated with spores suspended in sterile distilled water served as control. Culture plates were in triplicates for each treatment. The mean radial growth of fungus was calculated and the standard deviation of mean calculated. Data were analyzed statistically using the student t-test at a probability level of 0.05.

No growth was observed on treated cultures until day 2, whereas there was growth in the control from day 1 onward (**Table 1A**). Whereas 90% methanol allowed only little growth for the strains of *Aspergillus* and *Penicillium* treated for 3 min, no growth occurred for 5 and 10 min exposure on day 3 (**Table 1B-C**). There was no growth at all in the sodium hypochlorite-treated cultures. The length of exposure repressed growth in the order 10 > 5 > 3 min. Also, the higher the concentration of alcohol the greater the reduction in growth. The difference in growth between treated and control was significant for both fungi, especially on day 6.

The results presented here have shown that spores of *A. fumigatus* and *Penicillium* sp. could still germinate and grow after some days. Therefore, if present in cultures they would pose a threat to the successful isolation of pure cultures of desired fungal organisms. If the organisms to be grown are faster in growth than these two fungi the faster colonization of the growth medium by the former may suppress the germination and subsequent growth of *A. fumigatus* and *Penicillium*. It is evident from this report that ethanol and methanol (70% and 90%) will only be effective for surface sterilization where the desired fungus is faster-growing than the contaminating *Aspergillus* and *Penicillium*. Although ethanol (90%) applied for10 min is also recommended, sodium hypochlorite is the ideal disinfectant to use.

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Table 1. Mean mycelial radial growth of *Aspergillus fumigatus* (*Af*) and *Penicillium* sp. (*P*) after exposure to disinfectants and grown on SDA for 6 days at room temperature.

		Mean mycelial radial growth (cm ± sd) per day*					
Treatment		1	2	3	4	5	6
A	Control						
	Af	0.75+0.01	1.10±0.00	1.55±0.02	1.95±0.10	2.40±0.03	2.80^{a}±0.01
	P	0.35+0.00	0.60±0.00	0.90±0.00	1.15±0.02	1.75±0.02	2.05^{1}±0.03
	70% MeOH						
	Af	-	0.35±0.01	0.60±0.03	0.95±0.00	1.20^{b}±0.00	
	P	-	0.20±0.00	0.40±0.02	0.60±0.04	0.85^{2}±0.00	
	90% MeOH						
	Af	-	0.10±0.02	0.45±0.01	0.65±0.03	0.90^{b}±0.01	
	P	-	0.10±0.00	0.30±0.00	0.45±0.05	0.60^{2}±0.02	
	70% EtOH						
	Af	-	0.25±0.00	0.50±0.00	0.85±0.00	1.10^{b}±0.00	
	P	-	0.20±0.00	0.40±0.04	0.65±0.01	0.80^{2}±0.00	
	90% EtOH						
	Af	-	-	0.40±0.02	0.50±0.01	0.85^{b}±0.00	
	P	-	0.10±0.03	0.30±0.02	0.45±0.00	0.60^{2}±0.03	
B	Control						
	Af	0.45±0.01	0.85±0.00	1.20±0.04	1.65±0.01	2.05±0.03	2.20^{a}±0.02
	P	0.15±0.02	0.35±0.00	0.60±0.00	0.90±0.00	1.40±0.01	1.80^{1}±0.00
	70%MeOH						
	Af	-	-	-	0.40±0.00	0.75±0.03	0.95^{b}±0.01
	P	-	-	0.10±0.02	0.30±0.03	0.45±0.00	0.80^{2}±0.00
	90% MeOH						
	Af	-	-	-	0.20±0.01	0.45±0.00	0.65^{b}±0.00
	P	-	-	-	0.20±0.03	0.30±0.01	0.40^{3}±0.02
	70% EtOH						
	Af	-	-	1.0±0.04	0.35±0.02	0.65±0.00	0.90^{b}±0.00
	P	-	-	0.10±0.01	0.30±0.00	0.45±0.00	0.80^{2}±0.03
	90% EtOH						
	Af	-	-	-	0.30±0.02	0.40±0.00	0.60^{b}±0.00
	P	-	-	-	0.20±0.00	0.30±0.01	0.65^{2}±0.01
C	Control						
	Af	0.25±0.00	0.60±0.00	0.85±0.04	1.20±0.01	1.45±0.02	1.65^{a}±0.01
	P	-	0.20±0.02	0.40±0.00	0.60±0.01	1.00±0.00	1.35^{1}±0.00
	70%MeOH						
	Af	-	-	-	0.10±0.00	0.45±0.03	0.80^{b}±0.00
	P	-	-	-	0.20±0.04	0.15±0.01	0.35^{2}±0.01
	90% MeOH						
	Af	-	-	-	-	0.15±0.03	0.45^{c}±0.00
	P	-	-	-	0.10±0.02	0.20±0.00	0.30^{2}±0.03
	70% EtOH						
	Af	-	-	-	0.15±0.02	0.40±0.00	0.65^{b}±0.00
	P	-	-	-	0.15±0.00	0.30±0.02	0.40^{2}±0.01
	90% EtOH						
	Af	-	-	-	0.10±0.00	0.20±0.00	0.30^{c}±0.01
	P	-	-	-	0.15±0.00	0.30±0.02	0.40^{2}±0.01

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* Figures and letters in superscript which are different along the same column are significantly different (p= 0.05). Exposure: A= 3 min, B= 5 min, C= 10 min. MeOH= Methanol; EtOH= Ethanol; sd= Standard deviation; - = No growth.