

Investigation into the Efficacy of Formic Acid Fumigation for Inactivating Clinically Isolated Filamentous Fungi

Kaixuan Yuan^{1,*}, Benshun Tian^{2,*}, Annan Zhan^{3,*}, Zhuoxi Chen¹, Long Ye¹, Yong Ling¹, Suiling Liu¹, Xuejiao Bai⁴, Yunhu Zhao¹

¹Department of Clinical Laboratory Medicine, Guangdong Provincial People's Hospital (Guangdong Academy of Medical Sciences), Southern Medical University, Guangzhou, Guangdong, People's Republic of China; ²Faculty of Materials & Manufacturing, Beijing University of Technology, Beijing, People's Republic of China; ³KingMed School of Laboratory Medicine, Guangzhou Medical University, Guangzhou, Guangdong, People's Republic of China; ⁴Administration Department of Nosocomial Infection, Guangdong Provincial People's Hospital (Guangdong Academy of Medical Sciences), Southern Medical University, Guangzhou, Guangdong, People's Republic of China

*These authors contributed equally to this work

Correspondence: Xuejiao Bai, Administration Department of Nosocomial Infection, Guangdong Provincial People's Hospital, (Guangdong Academy of Medical Sciences), Southern Medical University, Guangzhou, No. 106 Zhongshan 2nd Road, Yuexiu District, Guangzhou, Guangdong, 510080, People's Republic of China, Tel/Fax +86 13580501919, Email baixuejiao@gdph.org.cn; Yunhu Zhao, Department of Clinical Laboratory Medicine, Guangdong Provincial People's Hospital, (Guangdong Academy of Medical Sciences), Southern Medical University, No. 106 Zhongshan 2nd Road, Yuexiu District, Guangzhou, Guangdong, 510080, People's Republic of China, Tel/Fax +86 17688485425, Email zhaoyunhu@gdph.org.cn

Purpose: Inactivating filamentous fungi (molds) reduce toxin production and transmission. This study evaluated the efficacy of formic acid (FA) fumigation to identify optimal conditions and its effects on fungal morphology. We aim to develop an efficient inactivation protocol for molds and provide valuable insights into laboratory techniques for fungal inactivation.

Methods: Initially, 238 clinical isolates from 23 species of molds were evaluated for the inactivation efficacy of formic acid, formaldehyde, and peracetic acid. After identifying the optimal sterilizing agent, 188 isolates from 16 clinically significant species were tested. Four concentrations (30%, 50%, 70%, and 100%) and six-time intervals (4 to 14 hours) were tested to optimize fumigation conditions. Morphological changes were analyzed using *Aspergillus fumigatus* ATCC MYA-3626 as the standard strain.

Results: The inactivation rates of 238 molds fumigated with formic acid, formaldehyde, and peracetic acid for 24 hours were 99.16%, 89.08%, and 90.35% ($P < 0.05$), indicating that formic acid was more effective than formaldehyde and peracetic acid. Subsequently, 188 isolates from 16 species were fumigated with formic acid at concentrations of 30%, 50%, 70%, and 100% for 24 hours, resulting in inactivation rate of 73.9%, 98.4%, 100%, and 100% ($P < 0.05$). Inactivation rate increased significantly with longer exposure times: 51.1% at 4 hours, 80.9% at 6 hours, 89.4% at 8 hours, 94.7% at 10 hours, 100% at 12 hours, and 100% after 14 hours ($P < 0.05$). After fumigation, *A. fumigatus* ATCC MYA-3626 growth ceased. Electron microscopy revealed irregular folds and roughness on spore surfaces, as well as collapsed or dead spores.

Conclusion: Formic acid demonstrated superior inactivation efficacy compared to formaldehyde and peracetic acid. At a concentration of 70% and a fumigation duration of 12 hours, the inactivation of molds was most effective.

Keywords: formic acid, formaldehyde, peracetic acid, fumigation, filamentous fungi, inactivation

Introduction

Filamentous fungi (molds) are a common type of fungi found in various environments, including clinical and laboratory settings as well as natural surroundings.¹ Their strong ability to survive and spread through spores and hyphae make them prone to contaminate laboratory equipment, air, and surfaces. Prolonged inhalation of these spores can cause respiratory issues like allergies, asthma, sinusitis, and lung infections.^{2,3} Immunocompromised individuals are particularly vulnerable to invasive fungal diseases, which are associated with high rates of missed diagnosis, misdiagnosis, and mortality.⁴

Inactivating filamentous fungi can hinder their transmission, infection ability, and mycotoxin production.^{5,6} Prior to conducting operations involving filamentous fungi, such as molecular sequencing and teaching demonstrations, biosafety concerns must be addressed through ensuring inactivation to prevent contamination or infection. Currently, commonly used sterilization methods include formaldehyde fumigation, peracetic acid sterilization, autoclave sterilization, and ultraviolet irradiation.^{7–10} However, formaldehyde fumigation is mainly used for air disinfection in farms, is carcinogenic, allergenic, and mutagenic, leading to its gradual phase-out.¹¹ Peracetic acid sterilization is corrosive to metals, has a short shelf life, and is challenging to store.¹² Autoclaving demonstrates reduced efficacy when addressing microorganisms with specialized resistance mechanisms. UV irradiation has poor efficacy against filamentous fungi. Therefore, finding an efficient method for inactivating filamentous fungi is highly significant, both theoretically and practically.

Currently, contact and fumigation methods are predominantly employed for microbial inactivation.^{7,8} Fumigation involves applying gaseous or volatile chemicals (including fungicides) to effectively reduce microbial abundance and diversity.¹³ One study demonstrated that β -cyclodextrin, when used as a fumigant, successfully inhibited *Penicillium digitatum* on citrus.¹⁴ Additionally, another study found chemical fumigants effective in suppressing *Fusarium* on fruit trees.¹⁵ Xiong et al evaluated the inhibitory effects of 1-octen-3-ol on *Fusarium tricinctum* and *Fusarium oxysporum* using both contact and fumigation methods, concluding that fumigation provided superior antifungal efficacy at lower dosages.¹⁶

The molecular formula of formic acid (FA) is HCOOH, making it the simplest carboxylic acid with notable acidity and antibacterial properties. In nature, carpenter ants and bees, among others, utilize endogenously produced formic acid in resin as a defensive mechanism against pathogens.^{17,18} One study demonstrated that formic acid can inhibit the growth of *Saccharomyces cerevisiae* cells during alcohol fermentation.¹⁹ Another study found that low concentrations of formic acid and acetic acid induce programmed cell death in *Candida*.²⁰ Furthermore, formic acid can serve as a lysis agent in mass spectrometry for the identification of fungi. These findings underscore the pronounced antifungal and fungicidal properties of formic acid. However, it is important to note that inhaling high concentrations of formic acid vapor can irritate the respiratory tract, potentially resulting in symptoms such as a sore throat, coughing, and breathing difficulties.^{17,18,20} During routine operations, it is critical to wear appropriate personal protective equipment, including safety goggles and masks, ensure adequate ventilation, and strictly adhere to relevant safety protocols.

However, researches on formic acid predominantly emphasizes its applications in food science and agriculture, with limited studies addressing the inactivation of filamentous fungi in clinical laboratories worldwide. Additionally, there is a significant lack of research regarding the use of formic acid fumigation for fungal inactivation. The aim of our study is to develop an efficient inactivation protocol specifically for filamentous fungi, investigate the efficacy of formic acid fumigation against common clinical filamentous fungi, determine the optimal concentration and duration necessary for effective fumigation, and offer valuable insights into the laboratory fungal inactivation techniques and hospital infection prevention.

Materials and Methods

Strains Selection

This study collected a total of 238 clinically isolated strains of filamentous fungi from Guangdong Provincial People's Hospital between 2022 and 2023. All strains were identified by the morphology and VITEK MS (v3.2) analysis. Strains with inconsistent results or lacking VITEK MS data were further classified through molecular sequencing using ITS1-ITS4 primers. The isolates included 100 strains of *Aspergillus* spp., comprising 20 strains each of *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus*, and *Aspergillus niger*; 10 strains each of *Aspergillus nidulans* and *Aspergillus sydowii*. Additionally, there were 40 strains of Mucorales, including 10 strains each of *Rhizopus arrhizus*, *Rhizopus microsporus*, *Lichtheimia corymbifera*, and *Cunninghamella bertholletiae*. The *Fusarium* spp. group consisted of 40 strains, with 10 strains of *Fusarium solani*, 6 strains of *Fusarium proliferatum*, 5 strains each of *Fusarium verticillioides* and *Fusarium oxysporum*, and 4 strains of *Fusarium dimerum*. Furthermore, 30 strains belonged to *Penicillium/Paecilomyces* spp., including 10 strains of *Penicillium citrinum*, 5 strains each of *Purpureocillium lilacinum*, *Penicillium chermesinum*, *Penicillium oxalicum*, and *Paecilomyces variotii*. Lastly, 28 strains represented other filamentous fungi, specifically 10 strains of *Talaromyces marneffeii*, 10 strains of *Lomentospora prolificans*, and 8 strains of *Scedosporium apiospermum*.

Instruments and Reagents

A. fumigatus ATCC MYA-3626 (China General Microbiological Culture Collection Center, CGMCC), Sabouraud Dextrose Agar (SDA) (Jiangmen Kailin Trading Co., Ltd., Guangdong, China), normal saline (France BioMérieux Company), formic acid (Aladdin Biochemical Technology Co., Ltd., Shanghai, China), formaldehyde (Guangzhou Yishengbao Animal Pharmaceutical Co., Ltd., Guangdong, China), peracetic acid (Shenzhen Weikangshi Biotechnology Co., Ltd., Guangdong, China), disposable inoculation ring (Copanne Trading Co., Ltd., Shanghai, China), secondary biological safety cabinet (Jinan Xinbeixi Biotechnology Co., Ltd., Shandong, China), mold incubator (Guangzhou Sanyuan Technology Co., Ltd., Guangdong, China).

Preparation of Spore Suspension

A total of 238 strains stored at -80°C were revived on SDA plates and incubated at 28°C for 5 days. About 5 mL of sterile distilled water was added to the surface of each colony, and the spores on the agar surface were scraped off. The resulting spore suspensions were collected and filtered through sterilized absorbent cotton after thorough mixing. The spore suspensions were then adjusted to a final concentration of 1×10^6 CFU/mL using a hemocytometer.

Formic Acid Fumigation

First, 10 μL of prepared spore suspensions were aseptically transferred onto SDA plates and inoculated as single colonies. The SDA plates were then incubated at 28°C for 48 h to allow the colonies to grow adequately. Subsequently, the lid of each SDA plate was lifted within a biosafety cabinet, and 500 μL of formic acid solution was applied to the inner surface of each lid. As the negative control, 500 μL of sterile distilled water was added to the lid. All plates were inverted and incubated in a sealed container at 28°C for complete fumigation (Figure 1).

Inactivation Rate Among Three Chemical Agents

About 10 μL of spore suspensions were inoculated onto three separate SDA plates and incubated at 28°C for 48 hours. About 500 μL of formic acid, formaldehyde, and peracetic acid solution were added to the lid of each plate, with the name of chemical agents clearly marked. All plates were completely sealed, and subsequent fumigation was performed at 28°C for 24 hours.

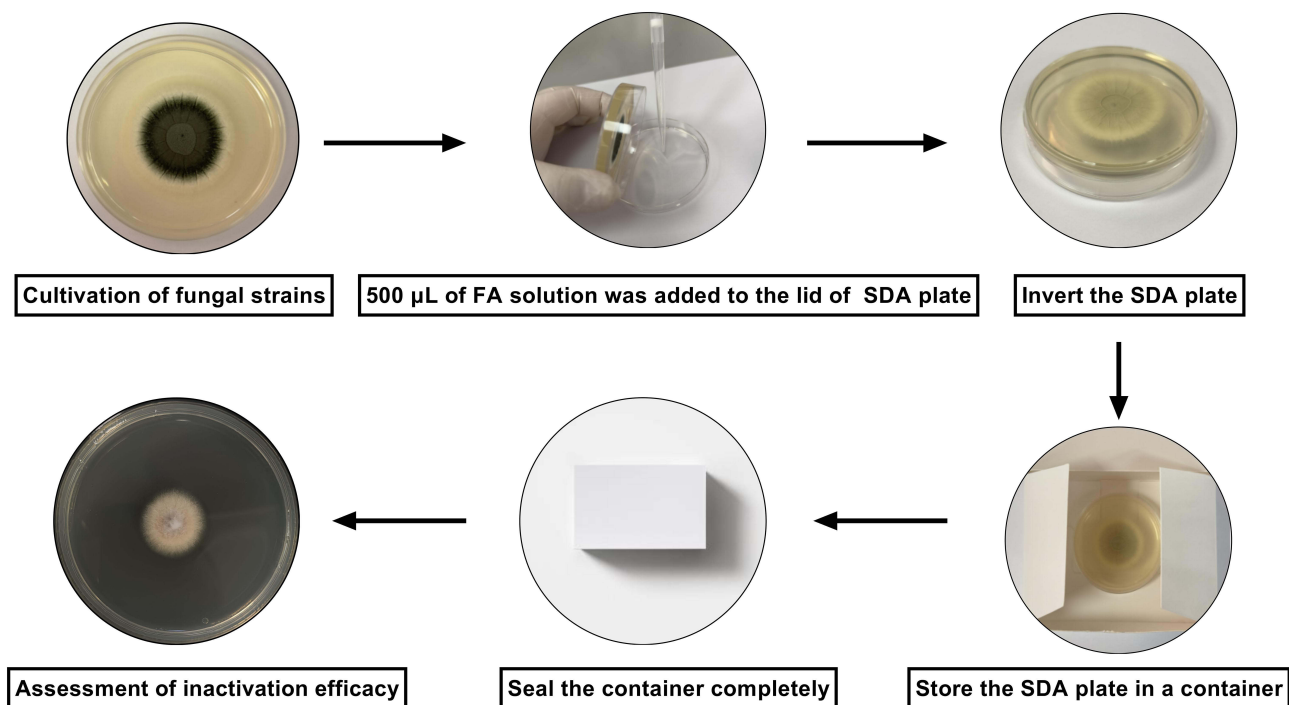


Figure 1 Formic acid fumigation procedures.

Optimization of Fumigation Concentration

About 10 μL of spore suspensions were inoculated onto four separate SDA plates, with each fungus inoculated on four plates as a single colony. The plates were incubated at 28°C for 48 hours. About 500 μL of pre-prepared formic acid solutions, with concentrations of 30%, 50%, 70%, and 100%, were added to the lid of each plate, with the respective concentrations clearly labeled. All plates were completely sealed, and subsequent fumigation was performed at 28°C for 24 hours.

Optimization of Fumigation Duration

About 10 μL of spore suspensions were inoculated onto six separate SDA plates, with each fungus inoculated on six plates as a single colony. The plates were then incubated at 28°C for 48 hours. About 500 μL of 70% formic acid solution was added to the lid of each plate, and the fumigation durations (4, 6, 8, 10, 12, and 14 hours) were marked. Subsequent fumigation was performed at 28°C.

Calculate the Inactivation Rate

After fumigation, the spores on the corresponding SDA plates were eluted, and the spore suspensions were adjusted to a final concentration of 1×10^6 CFU/mL. The suspensions were thoroughly mixed, and 10 μL was inoculated onto each plate. One colony was inoculated on one plate. The plates were incubated at 28°C for 5 days. The growth of colonies was observed and recorded. In this context, the inactivation efficacy was described using the inactivation rate, IR denotes the inactivation rate, N_{no_growth} represents the number of SDA plates exhibiting no colony growth, and N_{total} indicates the total number of SDA plates. The inactivation rates were calculated as follows:

$$IR(\%) = \left(\frac{N_{no_growth}}{N_{total}} \right) \times 100$$

Morphological Observation After Fumigation

Using *A. fumigatus* ATCC MYA-3626 as a model strain, conduct two replicate experiments. Following fumigation treatment with varying concentrations of formic acid and different exposure durations, the colony diameters were measured three times using a vernier caliper in a crosswise manner, and the changes in the average colony diameter were recorded. The colony color of *A. fumigatus* ATCC MYA-3626 before and after 70% formic acid fumigation for 12 hours, as well as the changes in its sporulation structures observed under light microscopy and electron microscopy, were compared. The negative control group strains received no formic acid treatment (sterile distilled water only) but underwent identical incubation and sealing procedures.

Statistical Analysis

The normality and variance tests were the first to be performed. Next, categorical variables were expressed as frequency and percentages and Intergroup comparisons were performed using the Chi-square (χ^2) tests or Fisher's exact tests. Variables with P-values < 0.05 were considered statistically significant. SPSS 26.0 software and GraphPad Prism 9.5.0 software were used for statistical analysis and data processing.

Results

Inactivation Rate Among Three Chemical Agents

The inactivation rates of filamentous fungi fumigated with formaldehyde, formic acid, and peracetic acid for 24 hours are shown in Table 1. The inactivation rates of 238 filamentous fungi fumigated with formaldehyde, formic acid, and peracetic acid for 24 hours were 89.08% (212/238), 99.16% (236/238), and 90.35% (215/238), respectively ($\chi^2 = 21.665$, $P < 0.001$), indicating a statistically significant difference among the treatments (Table 1). These results demonstrate that formic acid exhibits superior inactivation efficacy compared to both formaldehyde and peracetic acid, effectively inactivating the majority of filamentous fungi.

Further analysis of filamentous fungal species revealed that formic acid achieved a 100% inactivation rate against *Aspergillus*, *Fusarium*, *Penicillium/Paecilomyces*, *T. marneffei*, *L. prolificans*, and *S. apiospermum*. In contrast, formaldehyde exhibited the

Table 1 Inactivation Rates of Molds Fumigated with Three Chemical Agents for 24 Hours [n (%)]

Fungal Strains	n	Formaldehyde	Formic Acid	Peracetic Acid
Aspergillus spp.	100	88 (88.00)	100 (100.00)	90 (90.00)
<i>A. fumigatus</i>	20	20 (100.00)	20 (100.00)	20 (100.00)
<i>A. flavus</i>	20	19 (95.00)	20 (100.00)	17 (85.00)
<i>A. terreus</i>	20	18 (90.00)	20 (100.00)	19 (95.00)
<i>A. niger</i>	20	20 (100.00)	20 (100.00)	15 (75.00)
<i>A. sydowii</i>	10	2 (20.00)	10 (100.00)	9 (90.00)
<i>A. nidulans</i>	10	9 (90.00)	10 (100.00)	10 (100.00)
Mucorales	40	39 (97.50)	38 (95.00)	36 (90.00)
<i>R. arrhizus</i>	10	10 (100.00)	8 (80.00)	8 (80.00)
<i>R. microsporum</i>	10	9 (90.00)	10 (100.00)	10 (100.00)
<i>L. corymbifera</i>	10	10 (100.00)	10 (100.00)	10 (100.00)
<i>C. bertholletiae</i>	10	10 (100.00)	10 (100.00)	8 (80.00)
Fusarium spp.	40	29 (72.50)	40 (100.00)	38 (95.00)
<i>F. solani</i>	20	16 (80.00)	20 (100.00)	18 (90.00)
<i>F. proliferatum</i>	6	5 (83.33)	6 (100.00)	6 (100.00)
<i>F. verticillioides</i>	5	5 (100.00)	5 (100.00)	5 (100.00)
<i>F. oxysporum</i>	5	1 (20.00)	5 (100.00)	5 (100.00)
<i>F. dimerum</i>	4	2 (50.00)	4 (100.00)	4 (100.00)
Penicillium/Paecilomyces spp.	30	28 (93.33)	30 (100.00)	27 (90.00)
<i>P. citrinum</i>	10	10 (100.00)	10 (100.00)	8 (80.00)
<i>P. lilacinum</i>	5	5 (100.00)	5 (100.00)	5 (100.00)
<i>P. chermesinum</i>	5	5 (100.00)	5 (100.00)	5 (100.00)
<i>P. oxalicum</i>	5	5 (100.00)	5 (100.00)	5 (100.00)
<i>P. variotii</i>	5	3 (60.00)	5 (100.00)	4 (80.00)
Others	28	28 (100.00)	28 (100.00)	24 (85.71)
<i>T. marneffei</i>	10	10 (100.00)	10 (100.00)	10 (100.00)
<i>L. prolificans</i>	10	10 (100.00)	10 (100.00)	6 (60.00)
<i>S. apiospermum</i>	8	8 (100.00)	8 (100.00)	8 (100.00)
Total	238	212 (89.08)	236 (99.16)	215 (90.35)

poorest inactivation effect on *Aspergillus* and *Fusarium*, with inactivation rates of 88% (88/100) and 72.5% (29/40), respectively ($P < 0.05$). Notably, the inactivation rates for *A. sydowii* and *F. oxysporum* were only 20%. Additionally, peracetic acid showed poor efficacy in inactivating *L. prolificans*, achieving an inactivation rate of only 60% (6/10) ($P < 0.05$) (Table 1).

Optimal Fumigation Concentration of Formic Acid

The inactivation rates of filamentous fungi after 24 hours fumigation at various formic acid concentrations are shown in Table 2. A total of 16 species and 188 strains of clinically significant filamentous fungi were selected and fumigated with formic acid concentrations of 30%, 50%, 70%, and 100% for a duration of 24 hours. The inactivation rates were 73.9% (139/188), 98.4% (185/188), 100% (188/188), and 100% (188/188), respectively ($\chi^2 = 143.293$, $P < 0.001$). These results indicate that the inactivation efficacy of formic acid against filamentous fungi increases with higher concentrations. Specifically, at a concentration of 30%, the inactivation rate was only 73.9%, while at 50%, it increased to 98.4% ($\chi^2 = 47.223$, $P < 0.001$), showing a statistically significant difference. At a concentration of 70% formic acid, all 188 strains (100%) were completely inactivated (Table 2).

For *Fusarium*, the inactivation rate was only 17.50% (7/40) at a formic acid concentration of 30%. However, when the concentration was increased to 50%, the inactivation rate significantly rose to 70% (28/40). At a concentration of 70%, complete inactivation was achieved ($\chi^2 = 59.520$, $P < 0.001$). In contrast, *T. marneffei*, *A. flavus*, *A. terreus*, *R. arrhizus*, *R. microsporum*, and *C. bertholletiae* exhibited lower resistance to formic acid, achieving full inactivation (100%) at a concentration of 30% (Table 2).

Table 2 Inactivation Rates of Molds After 24 Hours Fumigation at Various Formic Acid Concentrations [n (%)]

Fungal Strains	n	Concentrations of Formic Acid (%)			
		30	50	70	100
<i>Aspergillus</i> spp.	98	89 (90.80)	87 (97.75)	98 (100.00)	98 (100.00)
<i>A. fumigatus</i>	20	18 (90.00)	20 (100.00)	20 (100.00)	20 (100.00)
<i>A. flavus</i>	20	20 (100.00)	20 (100.00)	20 (100.00)	20 (100.00)
<i>A. terreus</i>	20	20 (100.00)	20 (100.00)	20 (100.00)	20 (100.00)
<i>A. niger</i>	18	18 (100.00)	17 (94.44)	18 (100.00)	18 (100.00)
<i>A. sydowii</i>	10	9 (90.00)	10 (100.00)	10 (100.00)	10 (100.00)
<i>A. nidulans</i>	10	4 (40.00)	10 (100.00)	10 (100.00)	10 (100.00)
Mucorales	40	33 (82.50)	40 (100.00)	40 (100.00)	40 (100.00)
<i>R. arrhizus</i>	10	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)
<i>R. microsporum</i>	10	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)
<i>L. corymbifera</i>	10	3 (30.00)	10 (100.00)	10 (100.00)	10 (100.00)
<i>C. bertholletiae</i>	10	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)
<i>Fusarium</i> spp.	40	7 (17.50)	28 (70.00)	40 (100.00)	40 (100.00)
<i>F. solani</i>	20	4 (50.00)	18 (90.00)	20 (100.00)	20 (100.00)
<i>F. proliferatum</i>	6	1 (16.67)	6 (100.00)	6 (100.00)	6 (100.00)
<i>F. verticillioides</i>	5	0 (0.00)	5 (100.00)	5 (100.00)	5 (100.00)
<i>F. oxysporum</i>	5	1 (20.00)	5 (100.00)	5 (100.00)	5 (100.00)
<i>F. dimerum</i>	4	1 (20.00)	4 (100.00)	4 (100.00)	4 (100.00)
<i>T. marneffei</i>	10	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)
Total	188	139 (73.94)	185 (98.40)	188 (100.00)	188 (100.00)

Optimal Fumigation Duration of Formic Acid

The inactivation rates of filamentous fungi by 70% formic acid fumigation for various durations are shown in Table 3. The inactivation rates of 188 filamentous fungi were observed to be 51.1% (96/188) at 4 hours of fumigation, increasing to 80.9% (152/188), 89.4% (168/188), 94.7% (178/188), 100% (188/188), and 100% (188/188) at 6, 8, 10, 12, and 14 hours, respectively ($\chi^2 = 248.865$, $P < 0.001$). The results indicate a significant increase in the inactivation rate of filamentous fungi with extended exposure time to formic acid fumigation. Notably, 94.7% of the tested fungi were inactivated within 10 hours, while complete inactivation was achieved after 12 hours (Table 3).

For *Aspergillus*, the inactivation rate was 34.69% (34/98) after 4 hours of exposure. Extending the inactivation time to 12 hours resulted in complete inactivation of all *Aspergillus* isolates ($\chi^2 = 95.030$, $P < 0.001$). For *L. corymbifera* and *C. bertholletiae*, complete inactivation (100%) was achieved within 4 hours of fumigation. The inactivation rate for *T. marneffeii* was 40.00% (4/10) at 4 hours. However, extending the inactivation time to 6 hours led to complete inactivation, demonstrating a significant inactivation effect ($P < 0.05$) (Table 3).

Effect of Fumigation on Morphology

Colony Changes

The standard strain of *A. fumigatus* ATCC MYA-3626 was used as the experimental strain. *A. fumigatus* ATCC MYA-3626 in the control group exhibited normal growth, progressively covering the entire surface of the SDA plate over time.

Table 3 Inactivation Rates of Molds by 70% Formic Acid Fumigation for Various Durations [n (%)]

Fungal Strains	n	Fumigation Durations of Formic Acid (Hours)					
		4	6	8	10	12	14
<i>Aspergillus</i> spp.	98	34 (34.69)	68 (69.39)	80 (81.63)	88 (89.80)	98 (100.00)	98 (100.00)
<i>A. fumigatus</i>	20	4 (50.00)	12 (60.00)	17 (85.00)	20 (100.00)	20 (100.00)	20 (100.00)
<i>A. flavus</i>	20	4 (50.00)	9 (45.00)	13 (65.00)	15 (75.00)	20 (100.00)	20 (100.00)
<i>A. terreus</i>	20	15 (75.00)	18 (90.00)	19 (85.00)	19 (85.00)	20 (100.00)	20 (100.00)
<i>A. niger</i>	18	2 (11.11)	10 (55.56)	11 (61.11)	14 (77.78)	18 (100.00)	18 (100.00)
<i>A. sydowii</i>	10	2 (20.00)	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)
<i>A. nidulans</i>	10	7 (70.00)	9 (90.00)	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)
Mucorales	40	36 (90.00)	39 (97.50)	40 (100.00)	40 (100.00)	40 (100.00)	40 (100.00)
<i>R. arrhizus</i>	10	9 (90.00)	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)
<i>R. microsporum</i>	10	7 (70.00)	9 (90.00)	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)
<i>L. corymbifera</i>	10	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)
<i>C. bertholletiae</i>	10	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)
<i>Fusarium</i> spp.	40	22 (55.00)	35 (87.50)	38 (95.00)	40 (100.00)	40 (100.00)	40 (100.00)
<i>F. solani</i>	20	9 (45.00)	16 (80.00)	18 (90.00)	20 (100.00)	20 (100.00)	20 (100.00)
<i>F. proliferatum</i>	6	5 (83.33)	6 (100.00)	6 (100.00)	6 (100.00)	6 (100.00)	6 (100.00)
<i>F. verticillioides</i>	5	4 (80.00)	5 (100.00)	5 (100.00)	5 (100.00)	5 (100.00)	5 (100.00)
<i>F. oxysporum</i>	5	1 (20.00)	5 (100.00)	5 (100.00)	5 (100.00)	5 (100.00)	5 (100.00)
<i>F. dimerum</i>	4	3 (75.00)	3 (75.00)	4 (100.00)	4 (100.00)	4 (100.00)	4 (100.00)
<i>T. marneffeii</i>	10	4 (40.00)	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)	10 (100.00)
Total	188	96 (51.06)	152 (80.85)	168 (89.36)	178 (94.68)	188 (100.00)	188 (100.00)

In contrast to the control group, the growth of *A. fumigatus* ATCC MYA-3626 in the experimental group had ceased entirely, with the aerial hyphae exhibiting poor growth. However, the morphology remained stable, and the color of the colony did not change, which did not hinder the color observation (Figure 2A). Compared to the control group, the colonies showed a slight increase in size after fumigation with 30% formic acid for 4, 6, 8 and 10 hours, but the colony diameter ceased to increase after 12 hours. Colonies also exhibited slight growth at 50%, 70%, and 100% concentrations for 4 and 6 hours, but the colony diameter stopped increasing after 8 h of fumigation (Figure 2B).

Morphological Changes in the Conidial Head

The standard strain of *A. fumigatus* ATCC MYA-3626 was used as the experimental strain. After 12-hour fumigating *A. fumigatus* ATCC MYA-3626 colonies incubated for 24 and 48 hours with different concentrations of formic acid, the conidial heads were examined microscopically. For colonies incubated for 24 hours, the conidial heads of *A. fumigatus* ATCC MYA-3626 were significantly smaller compared to the control group. Additionally, the vesicles, conidia, and phialides exhibited poor growth or detachment, which substantially hindered the identification of morphological characteristics. In contrast, for the colonies incubated for 48 hours, the size of the conidial heads was more consistent with that of the control group, and there was no significant interference with the observation and identification of *Aspergillus* morphology. The results are shown in Figure 3.

Electron Microscopic Observations of Conidial Head

The standard strain of *A. fumigatus* ATCC MYA-3626 was used as the experimental strain. In the control group, under electron microscopy, *A. fumigatus* ATCC MYA-3626 exhibited a complete conidial head with fully developed and smooth phialides, regular structural organization, and normal sporulation morphology (Figure 4A). The conidia were oval-shaped, with intact and well-defined cell walls (Figure 4B). In the experimental group, the cell walls of the phialides in *A. fumigatus* ATCC MYA-3626 lost their characteristic flask-shaped morphology, becoming collapsed and shrunken, while the vesicles also exhibited signs of shrinkage and atrophy (Figure 4C). Following fumigation, the conidial morphology was significantly disrupted; some spores displayed irregularly wrinkled and rough outer surfaces, while others were shrunken and ruptured, with visible remnants of the spore wall remaining post-rupture (Figure 4D). Electron microscopy clearly demonstrated that formic acid exerted a significant destructive effect on the spore cell walls of *A. fumigatus* ATCC MYA-3626, leading to spore damage and cell death.

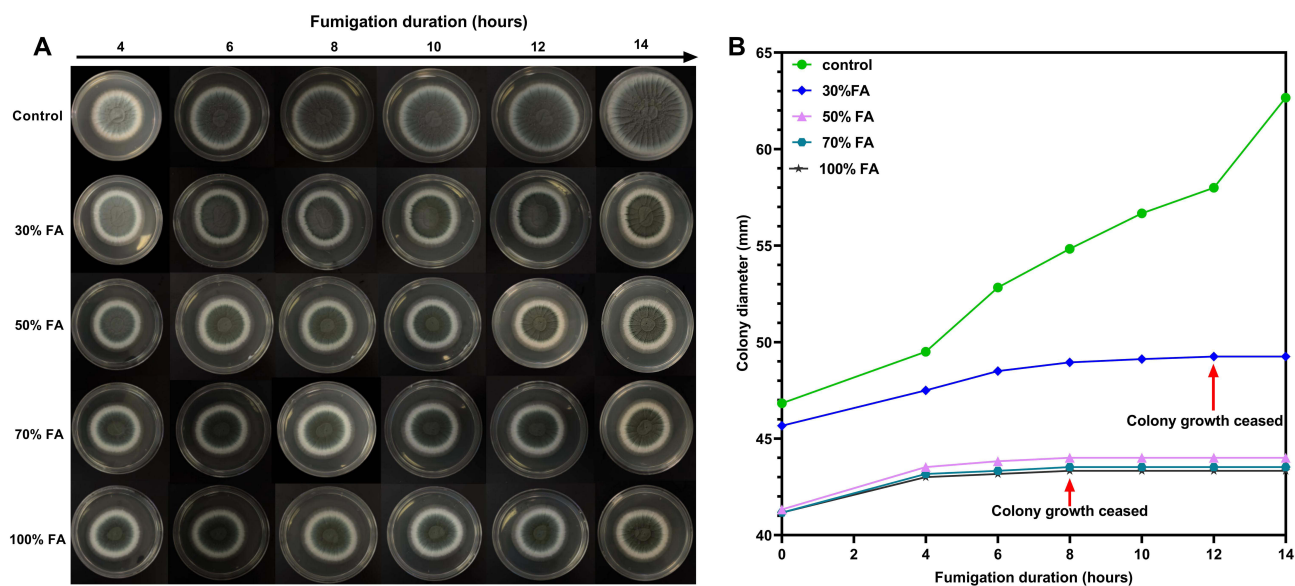


Figure 2 Changes in the diameter and color of *A. fumigatus* ATCC MYA-3626 colony at varying fumigation durations. (A) Colony morphology changes. (B) Colony diameter changes.

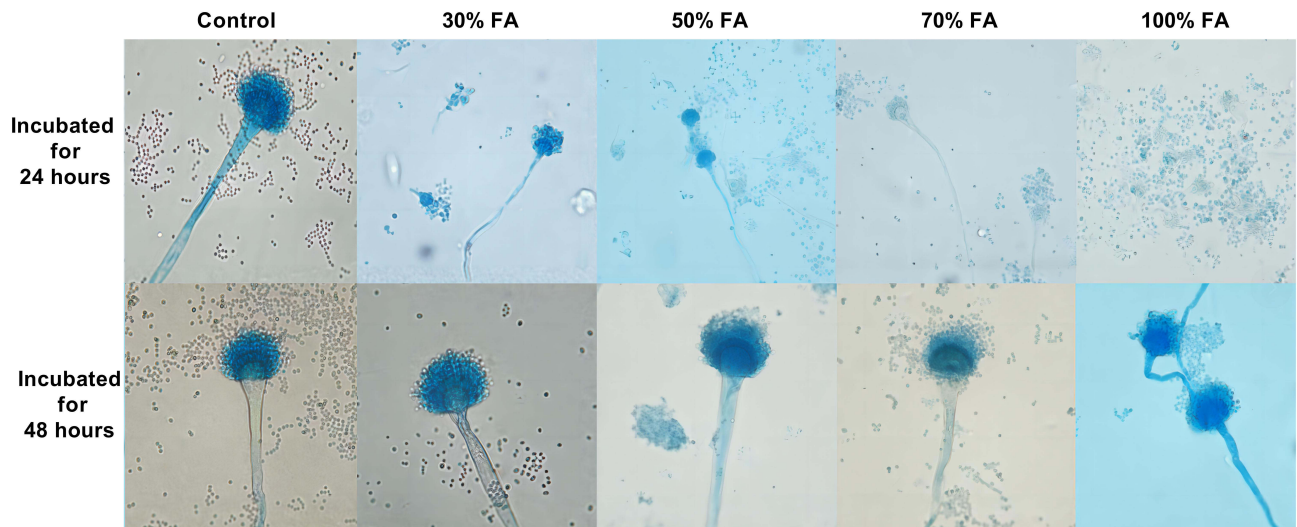


Figure 3 Effect of formic acid fumigation for 12 hours on conidial head of *A. fumigatus* ATCC MYA-3626 at varying concentrations (lactic acid phenolic cotton blue, 400 ×).

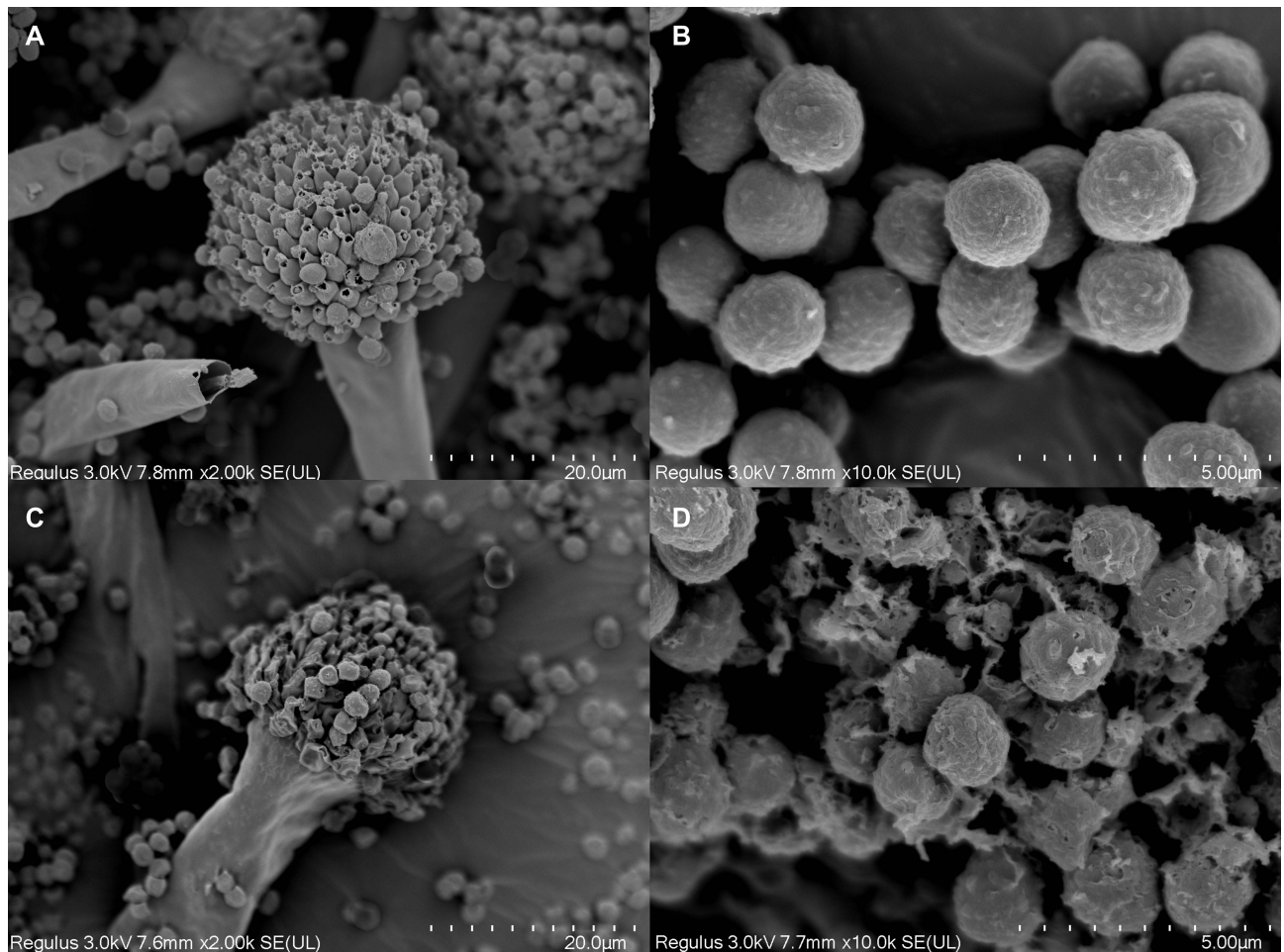


Figure 4 Comparison of conidial head and conidia morphology in *A. fumigatus* ATCC MYA-3626 between the control and experimental groups (electron microscope). (A) Conidial head (Control group). (B) Conidia (Control group). (C) Conidial head (Experimental group). (D) Conidia (Experimental group).

Discussion

The primary mode of transmission for filamentous fungal infections involves the airborne release and subsequent inhalation of conidia, predominantly affecting immunocompromised individuals.^{2,3,21,22} Interrupting this transmission pathway present a promising strategy for mitigating both the dissemination and infectivity of these fungi. However, there is limited literature regarding the efficacy of formic acid fumigation in deactivating filamentous fungi. Therefore, we conducted a study involving 188 strains from 16 commonly encountered clinical species to determine the optimal concentrations and exposure durations required for effective formic acid fumigation, as well as to evaluate its impact on fungal morphology.

The fumigation method involves the evaporation of chemical agents or fungicides to achieve microbial inhibition and sterilization.^{13,14} Li Y et al observed a significantly stronger inhibitory effect of formic acid on *S. cerevisiae* spores compared to acetic acid and levulinic acid.²³ Larsson et al demonstrated that even low concentrations of formic acid exhibit potent inhibitory activity against fungal spores.²⁴ In this study, we utilized fumigation to evaluate and compare the inactivation efficacy of formic acid, formaldehyde, and peracetic acid on filamentous fungi. Our findings indicated that formic acid demonstrated significantly higher efficacy (99.16%) in inactivating filamentous fungi compared to both formaldehyde (89.08%) and peracetic acid (90.35%). These results are consistent with the previous studies, collectively demonstrating that formic acid exhibits a significant antifungal effect.^{23,24} This phenomenon can be attributed to the lower pKa value and higher degree of dissociation of formic acid, which results in a greater production of hydrogen ions (H^+) and carboxylate anions ($-COO^-$) per unit mass.^{17–20} Consequently, this creates an ion concentration gradient between the interior and exterior of filamentous fungal cells, leading to intracellular dehydration, shrinkage, and potentially rupture, thereby impairing reproductive capability.⁶ Additionally, the carboxylate anion can penetrate the cell walls of filamentous fungi, disrupting the replication of intracellular genetic material or inhibiting enzyme activity.^{18,24} As a result, this effectively hinders the growth and reproduction of filamentous fungi. Compared to formic acid, formaldehyde demonstrated lower inactivation efficacy against *Aspergillus* and *Fusarium*, indicating suboptimal performance. Similarly, peracetic acid showed limited effectiveness against *L. prolificans*, achieving an inactivation rate of only 60%. This variability may be attributed to differences in cell wall composition and thickness among fungal species, as certain fungi possess robust and complex cell walls that confer resistance through their intrinsic antioxidant systems.²⁵ Additionally, the inadequate penetration of peracetic acid and formaldehyde into fungal cells further contributes to the reduced inactivation efficiency.^{25,26}

The optimal fumigation concentration of formic acid was further refined through a series of experiments conducted at four distinct concentration levels (30%, 50%, 70%, and 100%) over a 24-hour period, testing a total of 188 fungal strains. The experimental findings indicated that an increase in formic acid concentration resulted in a significant upward trend in the overall inactivation rate of filamentous fungi, thereby enhancing its inhibitory effect. At a concentration of 50%, the majority of filamentous fungi (98.40%) can be inactivated; however, some strains of *F. solani* and *A. niger* may remain viable. Complete inactivation of all filamentous fungi is achieved when the concentration is increased to 70%. The study conducted by ZENG et al also demonstrated that varying concentrations of formic acid exhibit differential inhibitory effects on fungal spore growth, with the inhibition effect increasing proportionally with concentration.²⁷ This phenomenon may be attributed to higher concentrations leading to a greater number of molecules and a more pronounced disparity in organic acid molecule concentration between filamentous fungal cells, thereby enhancing the diffusion of organic acids and consequently strengthening their fungicidal capabilities.^{17,18} Following treatment, the outer surface of the spores exhibited irregular wrinkling and roughness, with some spores undergoing shrinkage and rupture, resulting in a reduction in volume. Residual spores were observed post-rupture, indicating that formic acid likely disrupts the cell membrane and cell wall of filamentous fungal spores, leading to a loss of cellular integrity and subsequent death. Although 100% formic acid is fully effective against filamentous fungi, its high irritancy necessitates dilution to a concentration of 70% for practical fumigation applications.

To investigate the optimal duration for formic acid fumigation, we conducted a time-gradient treatment with durations of 4, 6, 8, 10, 12, and 14 hours. The experimental results demonstrated that at a concentration of 70%, the rate of inactivation progressively increased as the fumigation time extended, indicating a time-dependent inhibitory effect. This finding is consistent with the previous study that have reported the time-dependent inactivation of *Candida* spores by

formic acid.¹⁸ When the exposure time was extended to 20 hours, complete inactivation of all *Candida* spores was achieved. However, when the fumigation duration reached 8 hours, the rate of inactivation decelerated, and at 24 hours, complete inactivation was achieved.¹⁸ This phenomenon can be attributed to the volatilization and decomposition of formic acid in the surrounding environment, leading to a decline in effective concentration.¹⁹ Moreover, we observed varying degrees of fungal eradication by formic acid across different species. For instance, *L. corymbifera* and *C. bertholletiae* could be completely eliminated after exposure to 70% formic acid only for 4 hours, while *T. marneffeii* required 6 hours of fumigation. The most filamentous fungi (94.68%) can be effectively inactivated through formic acid fumigation over a period of 10 hours. Conversely, *A. niger*, *A. flavus*, and *A. terreus* necessitated an extended exposure time of 12 hours. Therefore, in clinical practice, it is crucial to select the appropriate fumigation duration and concentration based on different types of filamentous fungal species in order to achieve optimal inactivation efficacy. For *Aspergillus*, a fumigation duration of 12 hours is recommended when using 70% formic acid. For Mucorales, an 8-hour fumigation period is advised, and for *Fusarium*, a 10-hour fumigation is suggested.

Morphological identification following fumigation inactivation can effectively mitigate laboratory contamination and reduce the risk of operator infection. This study also addressed concerns regarding the impact of fumigation on colony morphology. After 48 hours of incubation, colonies subjected to fumigation exhibited significantly inhibited growth rate, maintaining fixed size and shape, with unchanged color, thereby preserving the integrity of colony color and morphology for observation. Under light microscopy, conidiophores remained intact and relatively uniform in size, ensuring that spore-producing structures could be observed without interference. However, when fumigation was applied after only 24 hours of incubation, conidial heads developed inadequately and were more prone to breakage. Electron microscopy revealed that the cell walls of phialides lost their characteristic flask-shaped morphology, collapsed, and shrank, while cell bodies became shriveled and vesicles appeared atrophied. Therefore, to ensure accurate morphological identification, it is inadvisable to fumigate and inactivate young fungi.

Our study developed a rapid and straightforward inactivation protocol, which provided valuable insights into laboratory techniques for fungal inactivation. It is important to note that the efficacy of this method is influenced by several factors, including the species of filamentous fungi, ambient temperature, and humidity. Future research will aim to further elucidate the mechanisms and factors associated with formic acid fumigation on filamentous fungi. Moreover, inhaling high concentrations of formic acid vapor can irritate the respiratory tract, leading to symptoms such as a sore throat, coughing, and breathing difficulties.^{17,18} When using formic acid for fumigation, it is essential to dilute it properly. Additionally, operators must adhere to safe dosage guidelines for formic acid and implement appropriate protective measures based on specific operational requirements.

Conclusions

In summary, our experimental results demonstrated that the fumigation method utilizing formic acid exhibited significantly greater inactivation efficacy against filamentous fungi compared to peracetic acid and formaldehyde. Furthermore, our data revealed that *T. marneffeii*, *Fusarium*, and Mucorales displayed heightened sensitivity to formic acid, while *Aspergillus* showed relatively diminished sensitivity. Specifically, when the concentration of formic acid was maintained at 70% and the fumigation duration was extended to 12 hours, the overall inactivation efficiency achieved its peak performance. This method is suitable for routine inactivation of filamentous fungal strains in clinical laboratories, offering operational simplicity and minimal impact on morphological observations. However, to achieve effective inactivation, consider the fungal species when setting fumigation time and concentration, and comply with safety regulations.

Ethical Form

This study was in accordance with the Research Ethics Committee of the Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences (KY2025-061-01). This study did not involve animal-related experiments, and no animal ethical requirements were needed.

Acknowledgments

We thank all our colleagues in Guangdong Provincial People's Hospital for their support of our project.

Funding

This study was supported by grants from Guangdong Basic and Applied Basic Research Foundation (2024A1515011037); National Natural Science Foundation of China (82302571).

Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Powers-Fletcher MV, Kendall BA, Griffin AT, et al. Filamentous Fungi. *Microbiol Spectr*. 2016;4(3). doi:10.1128/microbiolspec.DMIH2-00022015
2. Egbuta MA, Mwanza M, Babalola OO. health risks associated with exposure to filamentous fungi. *Int J Environ Res Public Health*. 2017;14(7):719. doi:10.3390/ijerph14070719
3. Borchers AT, Chang C, Eric Gershwin M. Mold and human health: a reality check. *Clin Rev Allergy Immunol*. 2017;52(3):305–322. doi:10.1007/s12016-017-8601-z
4. Denning DW. Global incidence and mortality of severe fungal disease. *Lancet Infect Dis*. 2024;24(7):e428–e438. doi:10.1016/S1473-3099(23)00692-8
5. Rodríguez M, Núñez F. Novel approaches to minimizing mycotoxin contamination. *Toxins*. 2020;12(4):216. doi:10.3390/toxins12040216
6. Rane B, Lacombe A, Guan J, et al. Reduction of *Aspergillus flavus* and aflatoxin on almond kernels using gaseous chlorine dioxide fumigation. *Food Chem*. 2023;402:134161. doi:10.1016/j.foodchem.2022.134161
7. Selby CM, Beer LC, Forga AJ, et al. Evaluation of the impact of formaldehyde fumigation during the hatching phase on contamination in the hatch cabinet and early performance in broiler chickens. *Poult Sci*. 2023;102(5):102584. doi:10.1016/j.psj.2023.102584
8. Noda M, Sakai Y, Sakaguchi Y, et al. Evaluation of low-temperature sterilization using hydrogen peroxide gas containing peracetic acid. *Biocontrol Sci*. 2020;25(4):185–191. doi:10.4265/bio.25.185
9. Sasaki JI, Imazato S. Autoclave sterilization of dental handpieces: a literature review. *J Prosthodont Res*. 2020;64(3):239–242. doi:10.1016/j.jpjor.2019.07.013
10. Li P, Ke X, Leng D, et al. High-intensity ultraviolet-c irradiation efficiently inactivates SARS-CoV-2 under typical cold chain temperature. *Food Environ Virol*. 2023;15(2):123–130. doi:10.1007/s12560-023-09552-5
11. Zuo L, Wu D, Deng M, et al. Simultaneous influence of light and CO₂ on phytoremediation performance and physiological response of plants to formaldehyde. *Environ Sci Pollut Res*. 2023;30:64191–64202. doi:10.1007/s11356-023-26969-4
12. Hosotani Y, Nakamura N, Kito H, et al. Effectiveness of sodium hypochlorite and peracetic acid for spoilage-causing molds on sweet potato slices. *Food Sci Technol Res*. 2023;29:257–267. doi:10.3136/fstr.fstr-d-22-00209
13. Castellano-Hinojosa A, Boyd NS, Strauss SL. Impact of fumigants on non-target soil microorganisms: a review. *J Hazard Mater*. 2022;427:128149. doi:10.1016/j.jhazmat.2021.128149
14. Y Liu, Weng L, Lin Y, et al. Carvacrol/ β -cyclodextrin inclusion complex as a fumigant to control decay caused by *Penicillium digitatum* on Shatangju mandarin slices. *Heliyon*. 2023;9(8):e18804. doi:10.1016/j.heliyon.2023.e18804
15. Jiang W, Chen R, Zhao L, et al. Chemical fumigants control apple replant disease: microbial community structure-mediated inhibition of *Fusarium* and degradation of phenolic acids. *J Hazard Mater*. 2022;440:129786. doi:10.1016/j.jhazmat.2022.129786
16. Xiong C, Li Q, Li S, et al. *In vitro* antimicrobial activities and mechanism of 1-octen-3-ol against food-related bacteria and pathogenic Fungi. *J Oleo Sci*. 2017;66(9):1041–1049. doi:10.5650/jos.ess16196
17. Ricke SC, Ditteo DK, Richardson KE. Formic acid as an antimicrobial for poultry production: a review. *Front Vet Sci*. 2020;7:563. doi:10.3389/fvets.2020.00563
18. Brutsch T, Jaffuel G, Vallat A, et al. Wood ants produce a potent antimicrobial agent by applying formic acid on tree-collected resin. *Ecol Evol*. 2017;7(7):2249–2254. doi:10.1002/ece3.2834
19. Zeng L, Huang J, Feng P, et al. Transcriptomic analysis of formic acid stress response in *Saccharomyces cerevisiae*. *World J Microbiol Biotechnol*. 2022;38(2):34. doi:10.1007/s11274-021-03222-z
20. Lastauskienė E, Zinkevičienė A, Girkontaitė I, et al. Formic acid and acetic acid induce a programmed cell death in pathogenic *Candida* species. *Curr Microbiol*. 2014;69(3):303–310. doi:10.1007/s00284-014-0585-9
21. Heung LJ, Wiesner DL, Wang K, et al. Immunity to fungi in the lung. *Semin Immunol*. 2023;66:101728. doi:10.1016/j.smim.2023.101728
22. den Nest M V, Wagner G, Riesenhuber M, et al. filamentous fungal infections in a tertiary care setting: epidemiology and clinical outcome. *J Fungi*. 2021;7(1):40. doi:10.3390/jof7010040
23. Li Y-C, Gou Z-X, Liu Z-S, Tang Y-Q, Akamatsu T, Kida K. Synergistic effects of TAL1 over-expression and PHO13 deletion on the weak acid inhibition of xylose fermentation by industrial *Saccharomyces cerevisiae* strain. *Biotechnol. Lett*. 2014;36(10):2011–2021. doi:10.1007/s10529-014-1581-7
24. Larsson S, Palmqvist E, Hahn H, et al. The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. *Enzyme Microb Technol*. 1999;24(3/4):151–159. doi:10.1016/j.palaeo.2005.05.015
25. Lin W, Zuo J, Li K, et al. Pre-exposure of peracetic acid enhances its subsequent combination with ultraviolet for the inactivation of fungal spores: efficiency, mechanisms, and implications. *Water Res*. 2023;229:119404. doi:10.1016/j.watres.2022.119404
26. Mchale CM, Smith MT, Zhang L. Application of toxicogenomic profiling to evaluate effects of benzene and formaldehyde: from yeast to human. *Ann N Y Acad Sci*. 2014;1310(1):74–83. doi:10.1111/nyas.12382
27. Zeng LJ, Huang JX, Feng PX, et al. Cytotoxicity of formic acid to *Saccharomyces cerevisiae*. *Food Sci*. 2022;43(6):125–131. doi:10.7506/spkx1002-6630-20210217-176

Infection and Drug Resistance

Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/infection-and-drug-resistance-journal>

Dovepress
Taylor & Francis Group