International Journal of Food Microbiology 289 (2019) 72–76

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Sensitivity of food spoilage fungi to a smoke generator sanitizer

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ARTICLE INFO

Keywords:
Air sanitization
Antifungal action
Food and environment hygiene

Chemical compound studied in this article:
Orthophenylphenol

ABSTRACT

Smoke generator sanitizers are easy to handle and can access to hard-to-reach places. They are a promising alternative for controlling food and air borne fungi, which are known to cause losses in the bakery, meat, and dairy industries. Therefore, the present study aimed to evaluate the efficiency of a smoke generator sanitizer based on orthophenylphenol against ten fungal species relevant to food spoilage. The tests were carried out according to the norms by the French protocol NF-T-72281, with adaptations specific for disinfectants diffused in the air. The tests were performed in an enclosed room of approximately 32 m^3. *Aspergillus brasiliensis* (ATCC 16404), *Candida albicans* (ATCC 10231), *Aspergillus flavus* (ATCC 9643), *Aspergillus chevalieri* (IMI 211382), *Cladosporium cladosporioides* (IMI 158517), *Lichtheima corymbifera* (CCT 4485), *Mucor hiemalis* (CCT 4561), *Penicillium commune* (CCT 7683), *Penicillium polonicum* (NGT 33/12), and *Penicillium roqueforti* (IMI 217568) were exposed to the smoke generator sanitizer for 7 h. The product was efficient against *C. albicans* and *C. cladosporioides*, although it was unable to reduce 4 log of the other tested species. The variable sensitivity of the fungal species to the sanitizer emphasizes the importance of confronting a target microorganism (causing problems in a specific food industry) with the sanitizer aiming to control it and obtain satisfactory results in hygiene programs.

1. Introduction

Fungi are widely distributed in the environment and can be found in the air, water, and soil. In order to adapt to their surroundings, these microorganisms must have different parameters of multiplication, sporulation capacity and dissemination (Pitt and Hocking, 2009). Airborne fungi are dispersed in the air in the form of small propagules and spread through airflow in the production environments of food industry facilities. This action is facilitated by the activity of collaborators, communication between distinct rooms, floor drains, and the ventilation system. These fungal particles, which are mostly spores, act as a contamination source at the location in which they settle in (Hedrick and Heldman, 1969).

After contaminating the food, the multiplication of these microorganisms can lead to product spoilage, promoting considerable economic losses, and in some cases, even pose risks to consumer health (Filtenborg et al., 1996; Pitt and Hocking, 2009; Samson et al., 2004). However, the negative economic impact can be minimized by contamination prevention and attention to the factors that enable microbial multiplication in food products (Asefa et al., 2010). Adopting hygienic-sanitary measures, such as effective cleaning and sanitization methods, can reduce the contamination of surfaces and the environment to adequate levels (Kuaye, 2017). It is well known that clean environments have reduced microbial counts and, therefore, less initial microbial load in processed foods, which extends their shelf life.

Smoke generator sanitizers are easy to handle, can access hard-to-reach places, and exert their effect during the exposure period while leaving little or no residue. These agents can be an important alternative for microbial control in food industries (Sholberg et al., 2004). In general, there is great concern to prevent bacterial development due to food safety issues. On the other hand, fungi are commonly identified just when the problem reaches considerable magnitudes, which results in food waste and economic losses (Dagnas et al., 2017; Garnier et al., 2017). Aerial dispersion of spores is considered a crucial point in controlling food spoilage fungi (Samson et al., 2004). Smoke generator agents are usually cheap and easy to use, making them a viable alternative to the bakery, meat, and dairy industries. These industries are the most affected by fungal deterioration, being air contamination a critical point for spore dissemination (Chitarra and Chitarra, 2005).

Phenolic sanitizers, such as orthophenylphenol (OPP) ($C_{12}H_{10}O$),

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https://doi.org/10.1016/j.ijfoodmicro.2018.09.004
Received 25 May 2018; Received in revised form 4 September 2018; Accepted 5 September 2018
Available online 06 September 2018
0168-1605/

are broad-spectrum, low-toxicity germicides, and their action is not impaired by the presence of organic matter. In addition to their recommended use on floors, footbaths, and having little residual activity (Grezzi, 2008), phenolic sanitizers can be used to decontaminate ambient air if used as smoke generator agents.

In Brazil, the use of orthophenylphenol-based disinfectant agents is authorized to sanitize food-processing environments and related industries. Moreover, the disinfectant tested in the present study is the first authorized in the country for this purpose (ANVISA (National Health Surveillance Agency), 2013). Orthophenylphenol-based disinfectant agents should be applied to food industry environments without the presence of food, even if no significant residual effects on citrus fruit are observed and they are below the residual limits recommended by Codex Alimentarius (10 mg Kg^{-1}) and the European Union (5 mg Kg^{-1}) (Besil et al., 2016). Orthophenylphenol exhibited low acute toxicity in animal experiments (Bomhard et al., 2002). The International Agency for Research on Cancer (IARC) classifies OPP as belonging to Group 3 (not classifiable as to its carcinogenicity to humans) (IARC (International Agency for Research on Cancer), 1999). Moreover, this organic compound is known to cause irritation to the skin, eye, and respiratory tract (PubChem, 2018), and, as a result, it should be manipulated with caution.

In countries close to Brazil, such as Uruguay, OPP or its sodium salt are used as a specific post-harvest fungicide (Besil et al., 2016). In other countries, such as the United States and European Union (EU) countries, this compound is most commonly used to decontaminate hospital areas. Some EU countries often employ this agent as a pesticide and preservative for citrus fruit and vegetables because of its efficacy as a biocide against bacteria, fungi, and yeasts (CDC, 2018; FAO, 1999; Coelhan et al., 2006).

Data on the antifungal activity of smoke generator agents against fungal species involved with food spoilage were not found in the literature. Therefore, this study aimed to verify the efficiency of an OPP-based fumigant sanitizer dispersed, in the air in the form of dry smoke, against fungal species present in the air of food industries and commonly involved in the deterioration of food products.

2. Materials and methods

2.1. Smoke generator sanitizer, recommended concentrations, and test room

The tests were performed in an enclosed room, which was exclusively used and prepared for this test, of approximately 32 m^3.

The product tested was an OPP 15% w/w smoke generator disinfectant. This product is commercialized in Brazil and authorized to sanitize food industry environments without the presence of food or raw materials. The product is presented in a cylindrical tin packaging format. Firing of the foot fumigation chamber was done by removing the seal and wick detachment. Then, it was lit and the smoke immediately dispersed, which quickly covered all the space of the test room. The sanitizer can was lit on the floor according to instructions in the manufacturer's manual.

The manufacturer indicates an OPP concentration of 1 g of product per m^3 of for this type of utility and exposure time of 7 h (the place of application must remain closed for 7 h and, once opened, the environment must be ventilated for at least 15 min before personal entrance). It is also recommended to clean the surfaces and equipment before reusing them.

2.2. Microorganisms used and standardization of the initial inoculum

Ten strains (2 standard strains for the sanitizing tests: *Aspergillus brasiliensis* (ATCC 16404) and *Candida albicans* (ATCC 10231); and 8 strains commonly related to ambient air and food product spoilage: *Aspergillus flavus* (ATCC 9643); *Aspergillus chevalieri* (Syn. *Eurotium chevalieri)* (IMI 211382); *Cladosporium cladosporioides* (IMI 158517); *Lichtheima corymbifera* (Syn. *Absidia corymbifera)* (CCT 4485); *Mucor hiemalis* (CCT 4561); *Penicillium commune* (CCT 7683); *Penicillium polonicum* (NGT 33/12); *Penicillium roqueforti* (IMI 217568)) were used. For preservation and quality assurance of viable cells, all strains were lyophilized and kept under refrigeration during the experiment.

For the initial spore solution preparation, tubes containing Malt Extract Agar (MEA) [glucose, 20 g (Neon, São Paulo, Brazil); peptone, 1 g (Himedia, Mumbai, India); malt extract, 30 g (Bacto™, MD, USA); metal traces, 1 mL; distilled water, 1 L], were inoculated with each fungal strain, followed by incubation for 7 days at 25 °C. Spores were collected by scraping the mycelium using sterile aqueous solution of polysorbate 80% (0.05%). Spore concentration was standardized in 10^2, 10^3, 10^4, and 10^5 spores/mL to evaluate the neutralization efficiency of the sanitizing effect of the product and 10^8 spores/mL for *in vitro* efficacy of the antifungal activity of the smoke generator sanitizer. Neutralization efficiency was confirmed after dilution in 0.1% peptone water [peptone, 0.1 g (Himedia, Mumbai, India); distilled water, 1 L] a Neubauer chamber and confirmed by inoculation in plates containing MEA and incubation for 5 days at 25 °C.

2.3. Evaluation of in vitro antifungal efficacy of the OPP smoke generator

The tests were carried out following the norms of the French protocol NF-T-72281 (Norme Française, NF T 72-281, 2014), with adaptations specific for sanitizers diffused by air. The test is illustrated in Fig. 1.

As carriers of microorganisms, 304 stainless steel discs of 2 cm diameter (TSM inox®, Santa Maria, Brazil), which were previously treated with 5% Triton ™ X-100 (Sigma-Aldrich, USA) for 60 min to remove impurities, were used. The disks were rinsed and left immersed in 70% (v/v) isopropanol for 24 h (Neon, São Paulo, Brazil).

2.3.1. Neutralizer efficacy evaluation

A neutralization step was carried out to ensure that the action of the fumigant agent only occurred during the test exposure time. Neutralization was performed with a solution containing casein tryptone 1 g/L, sodium chloride 8.5 g/L, polysorbate 80% 5 g/L, and a denominated recovery liquid (Jaenisch et al., 2010; Norme Française, NF T 72-281, 2014).

Previous tests were performed to evaluate the neutralization efficiency of the sanitizing effect of the product, as indicated in NF-T-72281. Two discs not contaminated with the fungal inoculum were used for this test. The discs were exposed to the fumigant for 7 h in order for the sanitizer to impregnate them.

Then, the impregnated discs were immersed in 100 mL of the previously described neutralizing liquid (recovery liquid) for 5 min. A 1 mL aliquot of the homogenate was then added to the sterile Petri dishes, followed by 1 mL of the inoculum previously adjusted at 10^2, 10^3, 10^4, and 10^5 spores/mL. Finally, 20 mL of Malt Extract Agar (malt extract, food grade, 30 g/L, 15 g/L agar) was added, homogenized, and left to solidify (pour plating).

The plates were incubated at 25 °C for 5 days. Afterwards, the colonies were counted and the neutralization effectiveness of the results compared with the positive control while following the tests described below.

2.3.2. Evaluation of in vitro antifungal efficacy

The smoke generator sanitizer efficacy test was performed by inoculating discs with 50 μL of a suspension of fungal spores previously adjusted to 10^8 spores/mL followed by the addition of 0.05% reconstituted skim milk powder (Elegê, São Paulo, Brazil), which simulated the presence of organic matter present in the environment. Five discs were inoculated with the fungi to be tested in each experiment. Three discs were exposed to the agent and used to test the sanitizer action. The other two discs were not exposed to the sanitizer and used as positive control of fungal inoculum.