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Antifungal activity of peracetic acid against toxigenic fungal contaminants of maize and barley at the postharvest stage

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ABSTRACT

Contamination of maize and barley grain during storage by the toxigenic fungi *Aspergillus flavus* (*A. flavus*) and *Penicillium Verrucosum* (*P. verrucosum*) is both an economic and a public health problem, especially in less industrialized countries. Peracetic acid (PA) is a compound used for the disinfection of food and food contact surfaces. Unlike other disinfectants, it leaves no toxic residues and its decomposition products (CH_3COOH, O_2 and H_2O) are environmentally friendly. In order to apply PA to preserve maize and barley grain during storage, first, the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) against both fungi were determined in a 96-well sterile microplates. Then, the antifungal activity of the volatile PA was determined by depositing it on filter paper and Hydroxyethylcellulose (HEC) gels, which were placed in airtight 1L flasks together with PDA and maize and barley grain inoculated with both fungi. The MFC in liquid medium of *A. flavus* and *P. verrucosum* was 93.8 and 187.5 mg/L respectively. However, PA doses of 300 and 200 mg/L on HEC gels were required in order to observe significant antifungal and antimycotoxigenic activity in maize and barley grain inoculated with these fungi.

1. Introduction

Maize and barley are the raw materials used to produce various products for human and animal consumption. However, grain loss in the post-harvest stage is relatively high (Abass et al., 2014; Darfour & Rosentrater, 2020). In the coming years, demand for grains is expected to increase due to the increase in the world population (OECD-FAO, 2020), and climate change is likely to affect the production and yields of grain crops (Singano, Mvumi, & Stathers, 2019; Yu, Luo, Wang, & Feil, 2020). Many of these cereals, such as maize in Sub-Saharan Africa, support the diet and economy of many countries (Gitonga, De Groote, Kassie, & Tefera, 2013).

Working on effective post-harvest technologies is one solution to reduce grain waste and ensure that the entire world population has physical, economic, and sufficient access to safe and nutritious food. Although the post-harvest loss of maize grain can have multiple causes, it is crucial to consider the storage stage as a critical point (Dumont. Orsat & Raghavan, 2016).

During storage, maize and barley grain are susceptible to contamination by *Aspergillus flavus* (*A. flavus*) and *Penicillium Verrucosum* (*P. verrucosum*) which cause physical, organoleptic, and nutritional deterioration. *A. flavus* produces aflatoxin B_1 (AFB_1), whereas *P. verrucosum* produces Ochratoxin A (OTA). Both mycotoxins are secondary metabolites that are toxic to humans and animals because they are potentially carcinogenic, mutagenic, hepatotoxic, teratogenic substances that can cause alterations at the epigenetic level, growth retardation and effects on the reproductive system (Alshannaq & Yu, 2017). Such is their importance at the public health level that the EU passed the regulation (EU) No. 165/2010 of the Commission of February 26, 2010 and the regulation (EC) No. 1881/2006 of the Commission of December 19, 2006 (EC, 2006) that sets the maximum level of aflatoxin B1 and ochratoxin A that food must have in order to be marketed.

In order to minimize the economic loss, caused by these fungi during storage, authors have proposed different strategies such as the improvement of several hermetic storage technologies, the ecosystem approach with the help of mathematical models and the application of

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environmentally friendly antifungal compounds (García-Díaz, Patiño, Vázquez, & Gil-Serna, 2019; Garcia-Lara, García-Jaimes, & Ortíz-Islas, 2020; Jian & Jayas, 2012; Nazareth et al., 2019).

Peracetic acid (PA) is an organic peroxide obtained from the acetylation reaction of acetic acid with hydrogen peroxide. As a powerful oxidant, PA oxidizes membranes, proteins and enzymes of the microorganisms and spores (Leggett et al., 2015; OMRI, 2016; Wang, Yamaki, Kawai, & Yamazaki, 2020). PA is environmentally friendly because its degradation products are acetic acid and hydrogen peroxide; both substances are considered GRAS (Generally Recognized As Safe) by the Food and Drug Administration (FDA). This fact makes PA, a possible substitute for chlorine-based disinfectants since they generate toxic substances for humans and the environment (Hassaballah, Bhatt, Nyitrai, Dai, & Sassoubre, 2020). Other studies such as Nicolau-Lapeña et al. (2019) and Banach et al. (2020) support polylactic acid as an alternative to fruit and vegetable washing. However, data on the antifungal effect of PA as preservative of stored grain are scarce in the scientific literature.

The study goals were first, to determine the PA *in vitro* antifungal activity against *A. flavus* and *P. verrucosum*. Then, to evaluate the potential of PA as a fumigant to avoid the fungal growth and the production of AFB_1 and OTA on maize and barley grain, respectively. Finally, to test a new PA release methodology based on hydroxyethylcellulose (HEC) and to evaluate its antifungal and antimycotoxigenic activity in a laboratory-scale silo.

2. Material and methods

2.1. Chemicals and strains

PA was obtained from Fisher Scientific (New Hampshire, EE.U.U.) and HEC was obtained from Sigma-Aldrich (St. Louis, MO, USA). AFB_1, OTA (purity of all mycotoxins $>$ 99%), formic acid (analytical grade, purity $>$ 98%) and ammonium formate (analytical grade, purity $\geq$ 99.0%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol (LC-MS grade, purity $\geq$ 99.9%) was purchased from Fisher Scientific (Hudson, NH, USA). Deionized water ($<$18 MΩ cm resistivity) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Previously, water and chromatography solvents were filtered with a 0.45 μm cellulose filter from Scharlau (Barcelona, Spain). Mycotoxin calibration standards were prepared from a 1 mg/mL stock solution (1 mg mycotoxin dissolved in 1 mL methanol). Both the standards and the mycotoxin extracts were stored in the dark at −20 °C before use. Maize and barley were obtained from TotAgro (Barcelona, España).

The strain ITEM 8111 of *A. flavus* was obtained from the Microbial Culture Collection of the Institute of Sciences and Food Production (ISPA, Bari, Italy). *P. verrucosum* VTT D-01847 was obtained from the VTT Technical Research Centre of Finland (Espoo, Finland). Peptone water, PDA, and PDB medium were obtained from Liofilchem Bacteriology Products (Roseto Degli Abruzzi, Italy).

2.2. Inoculum preparation

The strains were defrosted from liquid medium PDB with 25% glycerol and were grown in solid medium PDA at 25 °C for 7 d. Conidia were then harvested adding 5 mL of peptone water on top of the plates and scraping the PDA to form a suspension. The suspensions were recovered and transferred to a falcon tube (50 mL). Finally, the conidia concentration was adjusted for each trial using a Neubauer chamber.

2.3. Antifungal activity tests on liquid medium

The assay was performed in 96-well sterile microplates, using the modified Siah, Deweer, Morand, Reignault, and Halama (2010) method. The first microplate column was used as a negative control, where 200 μL of PDB was added to confirm medium sterility. The second column served as a positive control, containing non-treated microorganisms. In the rest of the columns (3-12), 100 μL of PA at doses between 6 and 4000 mg/kg were deposited together with 100 μL of a $5{\cdot}10^4$ spores/mL suspension in PDB of the mycotoxigenic fungi described in section 2.1. These plates were made in triplicate and incubated at 25 °C for 72 h in the dark.

The lowest PA concentration, where no visible fungal growth was observed, was considered the minimum inhibitory concentration (MIC). Finally, 10 μL of each higher PA concentration was deposited on PDA Petri plates at 25 °C for 72 h in order to determine the lethal concentration or minimum fungicide concentration (MFC).

2.4. Volatile antifungal activity tests in solid medium

The volatile antifungal activity assay was performed according to Nazareth et al. (2016) with some modification. In this test two different PA application methodologies were tested: first, the addition in a filter paper for a quick release, and secondly, its preparation in a 22% HEC gel for a slower release over time. For the gel preparation, 3.3 g of HEC was added to 15 mL of distilled water. The volume of PA necessary to obtain the desired final concentrations of the fungicide compound was added to this solution.

In both cases, petri dishes (50 mm diameter) were prepared with sterile PDA according to the manufacturer's specifications (42 g of PDA per 1 L of distilled water) and were inoculated by depositing in the center of the dishes 10 μL of two spore solutions of $1{\cdot}10^4$ CFU/mL and $1{\cdot}10^5$ CFU/mL for each pair of fungi (*A. flavus* and *P. verrucosum*) following the procedure described in point 2.2. These plates, together with the plate with the treatment were deposited on a glass plate (14 cm $\times$ 6 cm) and introduced in 1 L glass jars (JUVASA, Spain) previously sterilized in a Selecta autoclave (Barcelona, Spain) at 120 °C during 21 min (Fig. 1).

Thirty volatile PA concentrations between 0.1 mg/L and 200 mg/L were tested. In all cases, controls with filter paper and HEC gels without PA were incorporated.

The jars were kept at 25 ± 1 °C for seven days with a daily observation of the fungal growth. On the last day, the mycelial growth of the treated plates was measured and compared with the control plates.

2.5. Volatile antifungal activity test on maize and barley

The system used in this test was similar to the one described in point 2.4, replacing the inoculated PDA plates with other Petri dishes containing 10 g of contaminated cereals with 1 mL of a suspension of *A. flavus* (maize) and *P. verrucosum* (barley) up to a final concentration of $1{\cdot}10^4$ conidia/g (Fig. 2). First, maize and barley grain were autoclaved at 121 °C for 21 min and dried at room temperature inside a level 2 security cabinet. In this test, only the HEC gel method was tested because of its greater similarity to a possible commercial application. Volatile PA concentrations tested were 50 mg/L, 100 mg/L, 200 mg/L and 300 mg/L. The controls were performed with HEC gels without any amount of PA. Finally, the jars were kept at 25 ± 1 °C for two weeks, and each week the fungal growth and the formation of AFB_1 (maize) and OTA (barley) were analyzed.

Along with this test, another similar test was carried out with the same doses of PA applied in HEC gel but in which the contaminated cereals were replaced by non-autoclaved cereals not contaminated with any fungus. These jars were also kept at 25 °C for two weeks, and the reduction of the natural microbiological load was analyzed every week.

2.6. Determination of the fungal population

After the incubation time, 10 g of each sample was transferred to a sterile plastic bag containing 90 mL of sterile peptone water (Oxoid, Madrid, Spain) and homogenized with a stomacher (IUL, Barcelona, Spain) during 30 s. The suspensions formed were serially diluted in

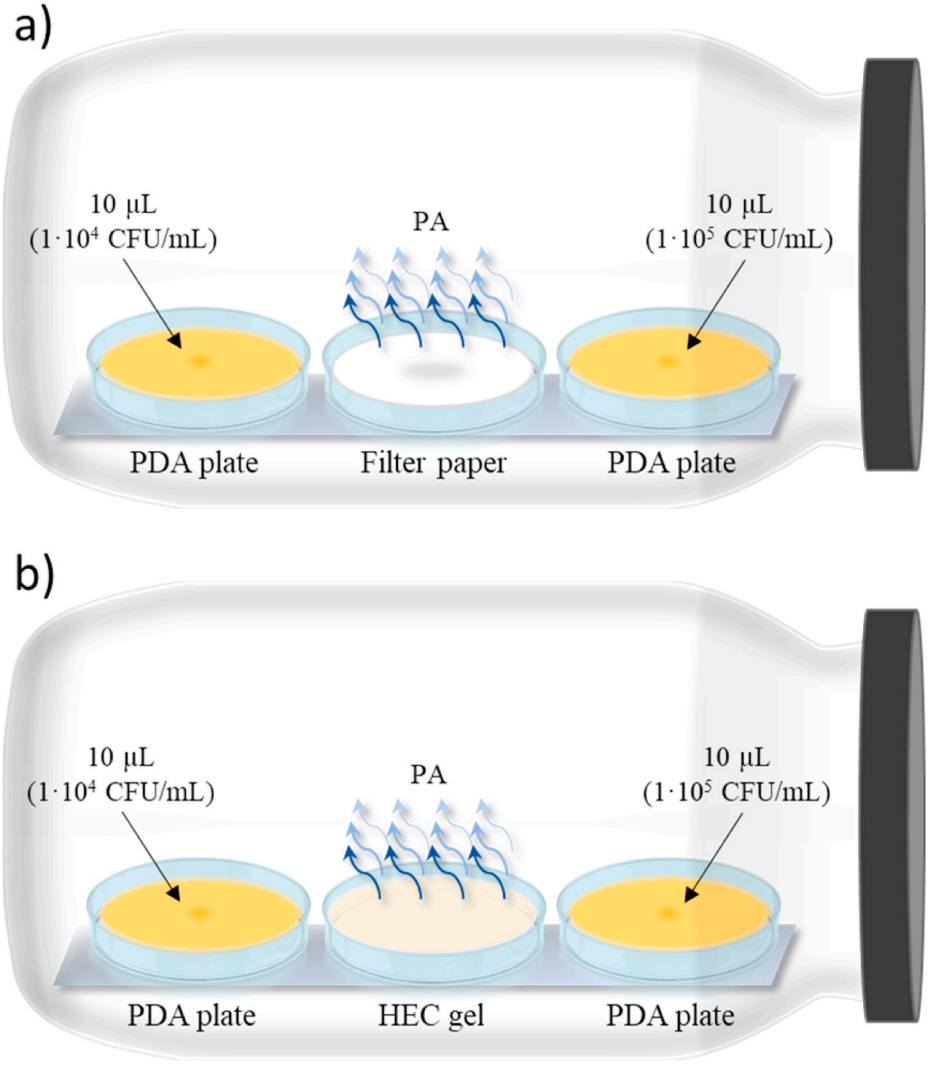

Fig. 1. System used to determine the volatile antifungal activity of PA against *A. flavus* ITEM 8111 and *P. verrucosum* VTT D-01847 in solid PDA medium using filter paper (a) and HEC gel (b).

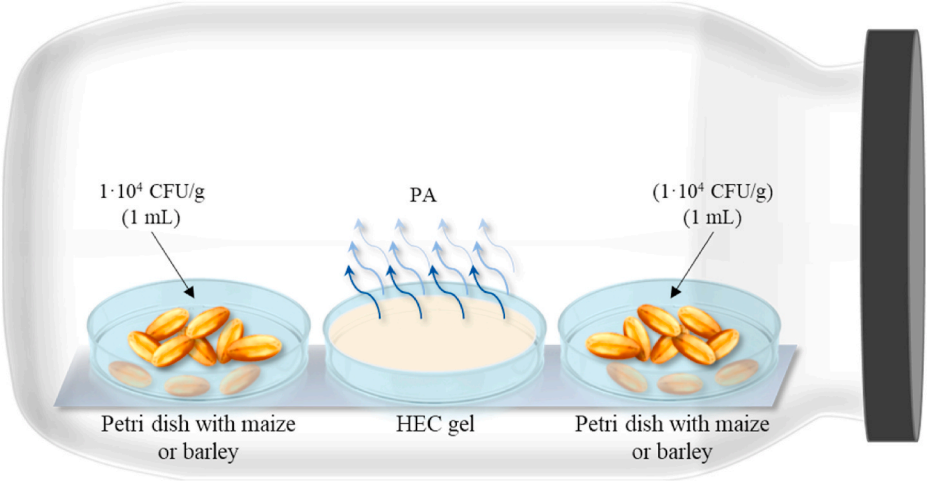

Fig. 2. System used to determine the volatile antifungal activity of PA against *A. flavus* ITEM 8111 and *P. verrucosum* VTT D-01847 in maize and barley grains using HEC gel.

sterile plastic tubes containing 0.1% of peptone water. After that, aliquots of 0.1 mL were plated on Petri dishes containing acidified potato dextrose agar (pH 3.5) (Insulab, Valencia, Spain) and the plates were incubated at 25 °C for 7 d before microbial counting. The results were expressed in a log of the colony-forming unit/g of cereal (log CFU/g). All analyses were conducted in triplicate.

2.7. Mycotoxin extraction and LC-MS/MS analysis

The extraction of mycotoxins was carried out following the method described by Serrano, Font, Mañes, and Ferrer (2013) with some modifications. Each cereal sample was crushed using a food grinder (Oster Classic Grinder 220e240 V, 50/60 Hz, 600 W, Oster, Valencia, Spain). The resulting particles were mixed, and three 5 g aliquots of each sample were taken in 50 mL plastic falcon tubes. 25 mL of methanol was added to each of these tubes and the samples were homogenized for 3 min by Ultra Ika T18 ultraturrax (Staufen, Germany) at 10000 rpm. The extract was centrifuged at 10670 G for 5 min at 5 °C, and the supernatant was transferred to a plastic flask and evaporated to dryness with a Büchi Rotavapor R-200 (Postfach, Switzerland). The obtained residue was resuspended in 5 mL of methanol, transferred to a 15 mL plastic falcon tube and evaporated with a nitrogen gas stream in a multi-sample Turbovap LV evaporator (Zymark, Hopkinton, MA, USA). Finally, the residue was reconstituted in 1 mL of methanol, filtered through a 13 mm/0.22 μm filter, and transferred to a 1 mL glass chromatography vial. The liquid-chromatography system consisted of an LC-20AD pump coupled to a 3200QTRAP mass spectrometer (Applied Biosystems, Foster City, CA, USA) using an ESI interface in positive ion mode. The mycotoxins were separated on a Gemini NX C18 column (150 × 2.0 mm I.D, 3.0 mm, Phenomenex, Palo Alto, CA, USA). The mobile phases were the solvent A (5 mM ammonium formate and 0.1% formic acid in water) and solvent B (5 mM ammonium formate and 0.1% formic acid in methanol) at a flow rate of 0.25 mL/min. The elution was carried out using a linear gradient from 0 to 14 min. The injection volume set was 20 mL, the nebulizer, the auxiliary, and the auxiliary gas were set at 55, 50, and 15 psi. The capillary temperature and the ion spray voltage were 550 °C and 5500 V, respectively. The ions transitions used for the mycotoxin identification and quantification were: m/z 313.1/241.3 and 284.9 for AFB_1 and m/z 404.3/102.1 and 358.1 for OTA.

This analytical method was validated by calculating linearity, recovery, repeatability, reproducibility, limits of detection (LOD) and limits of quantification (LOQ), and matrix effect for each mycotoxin analyzed. Linearity was evaluated using paired matrix calibrations in triplicate at concentrations between 5 and 500 μg/kg. All the mycotoxins showed good linearity in the working range, with resolution determination coefficients (R2) greater than 0.9922. LODs and LOQs were calculated by analyzing blank samples enriched with the standard mycotoxins; these parameters have been assessed as the lowest concentration of the molecules studied that showed a chromatographic peak at a signal-to-noise ratio (S/N) of 3 and 10 for LOD and LOQ, respectively. The value of the recovery was carried out in triplicate for three consecutive days using three addition levels: LOQ, 2 × LOQ, and 10 × LOQ. To calculate the matrix effect, the calibration slope from the matrix calibration curve was divided by the slope of the standard calibration curve and multiplied by 100. All these results are shown in Table 1.

Table 1
LODs, LOQs, recovery, and matrix effect (ME) (%) for AFB_1 and OTA in maize and barley.

Mycotoxin	LOD (μg/Kg)	LOQ (μg/Kg)	Recovery (%)	ME (%)
AFB_1	0.08	0.27	70.4	78.2
OTA	0.05	0.17	75.6	89.7

2.8. Statistical analysis

Data were statistically analyzed using the InfoStat software version 2008. The differences between groups were analyzed by one-way ANOVA, followed by the Tukey HSD post hoc test for multiple comparisons. The significance level was set at $p \leq 0.05$.

3. Results and discussion

3.1. Antifungal activity tests on liquid medium

The PA MIC for *A. flavus* was 125 mg/L, while a dose of 187.5 mg/L was necessary to reach the MFC. The PA MIC for *P. verrucosum* was 62.5 mg/L, while MFC was 93.8 mg/L. These results show that *P. verrucosum* is more sensitive to PA than *A. flavus*.

Kyanko, Russo, Fernández, and Pose (2010) have demonstrated the effectiveness of PA against several species of fungi of the genera *Penicillium* and *Aspergillus* at doses of 0.05%, 0.1% and 0.3% and a contact time of 30 min. Specifically, *A. flavus* presented a log reduction of 0.7 against an PA concentration of 0.3%. Bernardi et al. (2018), using the standards established by the European Committee for Standardization (CEN) (European Standard 13697, 2001) have demonstrated the effectiveness of PA against several species of fungi of the genera *Penicillium* and *Aspergillus* at doses of 0.15%, 1% and 3%. Specifically, *Penicillium comune* presented a log reduction of between 2 and 2.9 against PA concentration of 0.15% while for the same dose, the reduction of *Aspergillus brasiliensis* was between 1 and 1.9 log. Olivier, Stefanello, Gonçalves, Valle, and Venturini (2019), studied the antifungal activity of PA (0.15%, 1.5%, and 3%) against several strains of fungal species responsible for the deterioration of bakery products (genera *Penicillium* and *Aspergillus*). Specifically, at PA concentrations of 3%, the spores of *Penicillium roqueforti* and *Penicillium paneum* strains were reduced by 3–5 log and those of *Aspergillus pseudoglaucus* by 0.9 logs.

3.2. Volatile antifungal activity tests in solid medium

Semi-quantitative tests for evaluating the antifungal activity of PA using filter paper confirmed that the rapid volatilization of PA gaseous exerts a fungicidal action on *A. flavus* and *P. verrucosum*. As shown in Table 2, a concentration of 1 mg/L of volatilized PA can eliminate the growth of *A. flavus* on plates inoculated with ten μL of a concentration of $1{\cdot}10^4$ CFU/mL spores. The table also shows that 1.5 mg/L of volatilized PA can prevent the growth of *A. flavus* at an inoculum concentration of $1{\cdot}10^5$ CFU/mL. For *P. verrucosum*, the PA dose capable of eliminating fungal growth was 0.5 1 mg/L for the two inocula ($1{\cdot}10^4$ CFU/mL and $1{\cdot}10^5$ CFU/mL).

The use of PA as a volatile antifungal has not been studied yet, although other similar compounds have been. Ocak, Çelik, Özel, Korcan, and Konuk (2012) investigated the growth inhibition of 14 fungal species using volatile *Origanum hypericifolium* oil. The author placed a 5 mm diameter disk of PDA in Petri dishes with the fungal species to be studied and added 20 μL of the volatile oil in the lid of a Petri dish, wich was incubated at 20 °C. Higher doses were employed in that study, resulting in *A. flavus* inhibition, the most sensitive species, on the third day of treatment. In contrast, *P. verrucosum* grew after six days being the most resistant species of the studied fungi. In another study conducted by Ul Hassan, Al Thani, Alnaimi, Migheli, and Jaoua (2019), it was observed how exposing various fungi to volatile compounds released by *Bacillus licheniformis* managed to significantly reduce the growth of various fungi such as *A. flavus* and *P. verrucosum* and the production of aflatoxins (AFs) and OTA. However, the volatile compounds did not eliminate these fungi.

Concerting the use of HEC gel, the slower release of PA resulted in higher lethal doses (Table 3). A concentration of 10 mg/L of volatilized PA avoided the growth of *A. flavus* on plates inoculated with ten μL of a concentration of $1{\cdot}10^4$ CFU/mL spores, while one of 25 mg/L of

Table 2
Antifungal activities of volatile PA against two different inoculums of *A. flavus* ITEM 8111 and *P. verrucosum* VTT D-01847. The daily growth observed in the inoculated plates (+) and the percentage of reduction in the mycelial diameter at day 7.

PA (mg/L)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	% reduction (day 7)
A. flavus ($1{\cdot}10^4$ CFU/mL)								
Control	-	+	+	+	+	+	+	0.0%
0,25	-	+	+	+	+	+	+	5.0%
0,5	-	-	+	+	+	+	+	17.5%
0,75	-	-	-	+	+	+	+	22.5%
1–100	-	-	-	-	-	-	-	100.0%
***A. flavus* ($1{\cdot}10^5$ CFU/mL)**								
Control	-	+	+	+	+	+	+	0.0%
0,25	-	+	+	+	+	+	+	2.1%
0,5	-	+	+	+	+	+	+	5.0%
0,75	-	-	+	+	+	+	+	12.1%
1	-	-	+	+	+	+	+	15.0%
1,25	-	-	+	+	+	+	+	22.5%
1,5 -100	-	-	-	-	-	-	-	100.0%
***P. verrucosum* ($1{\cdot}10^4$ CFU/mL)**								
Control	-	+	+	+	+	+	+	0.0%
0,25	-	+	+	+	+	+	+	71.0%
0,5 -100	-	-	-	-	-	-	-	100.0%
***P. verrucosum* ($1{\cdot}10^5$ CFU/mL)**								
Control	-	+	+	+	+	+	+	0.0%
0,25	-	+	+	+	+	+	+	12.9%
0,5 -100	-	-	-	-	-	-	-	100.0%

Table 3
Antifungal activities of volatile PA in 22% HEC gel against two different inoculums of *A. flavus* ITEM 8111 and *P. verrucosum* VTT D-01847. The daily growth observed in the inoculated plates (+) and the percentage of reduction in the mycelial diameter at day 7.

PA (mg/L)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	% reduction (day 7)
A. flavus ($1{\cdot}10^4$ CFU/mL)								
Control	-	+	+	+	+	+	+	0.0%
1	-	+	+	+	+	+	+	11.8%
2,5	-	+	+	+	+	+	+	12.5%
5	-	-	+	+	+	+	+	13.2%
7,5	-	-	-	+	+	+	+	19.8%
10–100	-	-	-	-	-	-	-	100.0%
***A. flavus* ($1{\cdot}10^5$ CFU/mL)**								
Control	-	+	+	+	+	+	+	0%
1	-	+	+	+	+	+	+	1.0%
2,5	-	+	+	+	+	+	+	1.3%
5	-	+	+	+	+	+	+	2.6%
7,5	-	-	+	+	+	+	+	7.6%
10	-	-	-	+	+	+	+	20.3%
25–100	-	-	-	-	-	-	-	100.0%
***P. verrucosum* ($1{\cdot}10^4$ CFU/mL)**								
Control	-	+	+	+	+	+	+	0.0%
1	-	+	+	+	+	+	+	0.5%
2,5	-	+	+	+	+	+	+	14.0%
5	-	+	+	+	+	+	+	15.0%
7,5-100	-	-	-	-	-	-	-	100.0%
***P. verrucosum* ($1{\cdot}10^5$ CFU/mL)**								
Control	-	+	+	+	+	+	+	0.0%
1	-	+	+	+	+	+	+	5.0%
2,5	-	+	+	+	+	+	+	6.7%
5	-	+	+	+	+	+	+	15.0%
7,5	-	-	+	+	+	+	+	35.0%
10–100	-	-	-	-	-	-	-	100.0%

volatilized PA inhibited the growth of the inoculum concentration of $1{\cdot}10^5$ CFU/mL. Regarding *P. verrucosum*, PA concentration needed inhibit fungal growth was 7.5 mg/L wholly, and 10 mg/L for the samples inoculated whit $1{\cdot}10^4$ CFU/mL and $1{\cdot}10^5$ CFU/mL, respectively.

The use of gels with antimicrobial properties for food preservation is a new and not developed field of research. However, some studies showed how it could be a good alternative to classical preservatives against food altering and pathogenic microorganisms. Rao et al. (2020) proved how 0.40 mg/mL of a gel formed by carvacol encapsulation in ovalbumin nanoparticles managed to eliminate *Bacillus* cereus and *Salmonella* spores. Another study carried out by Paris, Ramírez-Corona, Palou, and López-Malo (2020) determined the effectiveness of encapsulation in sodium alginate gel of the essential oil *Cinnamomum zeylanicum* in the vapor phase (5% v/v) against the following fungi *Botrytis cinerea*, *Penicillium expansum*, *Alternaria alternata* or *Colletotrichum gloeosporioides*. The MIC values for each of these fungi were: 1.89 mg/L, 1.89 mg/L, 1.75 mg/L, 1.32 mg/L, respectively.

3.3. Volatile antifungal activity test on maize and barley

The results of the fungal growth of *A. flavus* on maize and *P. verrucosum* on barley treated with PA HEC gels at weeks 1 and 2 are shown in Fig. 3. In the case of *A. flavus*, after one week of treatment, a PA concentration of 100 mg/L inside the jar was able to reduce the fungal

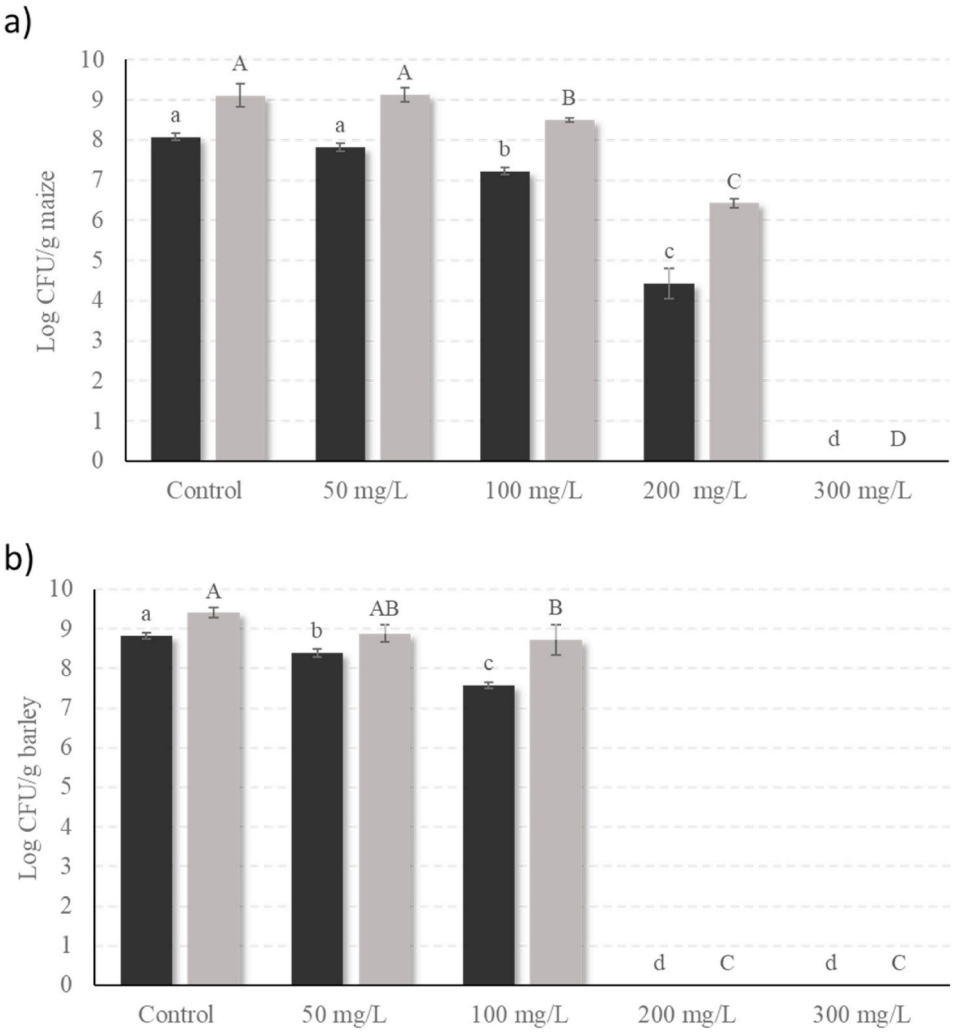

Fig. 3. Growth of *A. flavus* ITEM 8111 in maize (a) and *P. verrucosum* VTT D-01847 in barley (b) in a 1L jar, exposed to PA vapor by HEC gel after 1 week (dark grey) and 2 weeks (light grey) of incubation. Significant differences between treatments within each week are marked with different letters ($p \leq 0.05$).

population by 0.85 Log CFU/g (85.99%), 200 mg/L reduced it by 3.65 Log CFU/g (99.97%), and 300 mg/L eliminated the fungi. In week 2 the results were very similar, with a reduction of 0.67 Log CFU/g (78.42%) for 100 mg/L, a reduction of 2.75 Log CFU/g (99.82%) for 200 mg/L, and the complete disappearance of *A. flavus* at 300 mg/L of PA. In the case of *P. verrucosum*, after one week of treatment a PA concentration of 50 mg/L inside the jar was able to reduce the fungal population by 0.45 Log CFU/g (62.64%), 100 mg/L reduced it by 1.25 Log CFU/g (94.37%), and 200 mg/L eliminated the fungi. In week 2, the results were quite similar, a reduction of 0.52 Log CFU/g (68.15%) for 50 mg/L, 0.69 Log CFU/g (75.54%) for 100 mg/L, and 200 mg/L of PA completely inhibited *P. verrucosum* growth.

The reduction of AFB_1 on maize and OTA on barley treated with APA HEC gels at weeks 1 and 2 are shown in Fig. 4. In the case of maize inoculated with *A. flavus*, after one week the untreated control showed an AFB_1 concentration of 2010.7 μg/kg, while treatments with a PA concentration of 100 mg/L inside the jar could reduce AFB_1 by 77.57% (453.0 μg/kg), 200 mg/L by 92.42% (152.4 μg/kg) and 300 mg/L completely inhibited AFB_1 formation. At week 2, the results were similar, with an AFB_1 concentration in the untreated control of 2779 μg/kg and a reduction for the treatment of 100 mg/L of 47.8% (1050.4 μg/kg), 200 mg/L by 84.4% (313.9 μg/kg) and 300 mg/L completely inhibited AFB_1 formation. In the case of barley inoculated with *P. verrucosum*, none of the samples tested were detected as OTA after one week. In the second week, the untreated control showed an OTA concentration of 56.3 μg/kg, with no significant difference with the 50 mg/L PA treatment. In contrast, 100 mg/L PA reduced produced OTA in 92.42% (152.4 μg/kg), while 200 mg/L completely inhibited it. Comparison of the results of mycotoxin formation with those of fungal growth inhibition in the presence of PA indicates that the reduction of AFB_1 and OTA is explained by the elimination of toxigenic fungi.

The reduction of growth of toxigenic fungi and mycotoxins in food by using volatile compounds has been studied in substances other than PA. Ozone treatments have reduced the AFB_1 content in contaminated wheat by more than 80%. (Wang, Liu, Lin, & Cao, 2010). Essential oils (EO) have shown their efficacy as antifungal compounds and have been used to reduce mycotoxin synthesis in food. Reduction of AFs has been observed using 250 ppm of EO from *Thymus eriocalyx* (Rasooli & Owlia, 2005) and 450 ppm of EO from *Rosmarinus officinalis* (Rasooli et al., 2008). In the case of OTA, *Cinnamomum verum* EO requires doses above 200 ppm to inhibit its production (Hua et al., 2014). Finally, in a similar model to the one studied in this work, Quiles et al. (2019) treated maize contaminated with A. flavus with 500 ppm of allyl isothiocyanate (AITC) HEC gels, achieving a 98.51% reduction in AFB_1 production.

3.4. Volatile antifungal activity test in non-inoculated commercial maize

The results of reducing fungal contamination in naturally contaminated commercial maize treated with PA HEC gels at weeks 1 and 2 are shown in Fig. 5. After one week of treatment, a PA concentration of 50

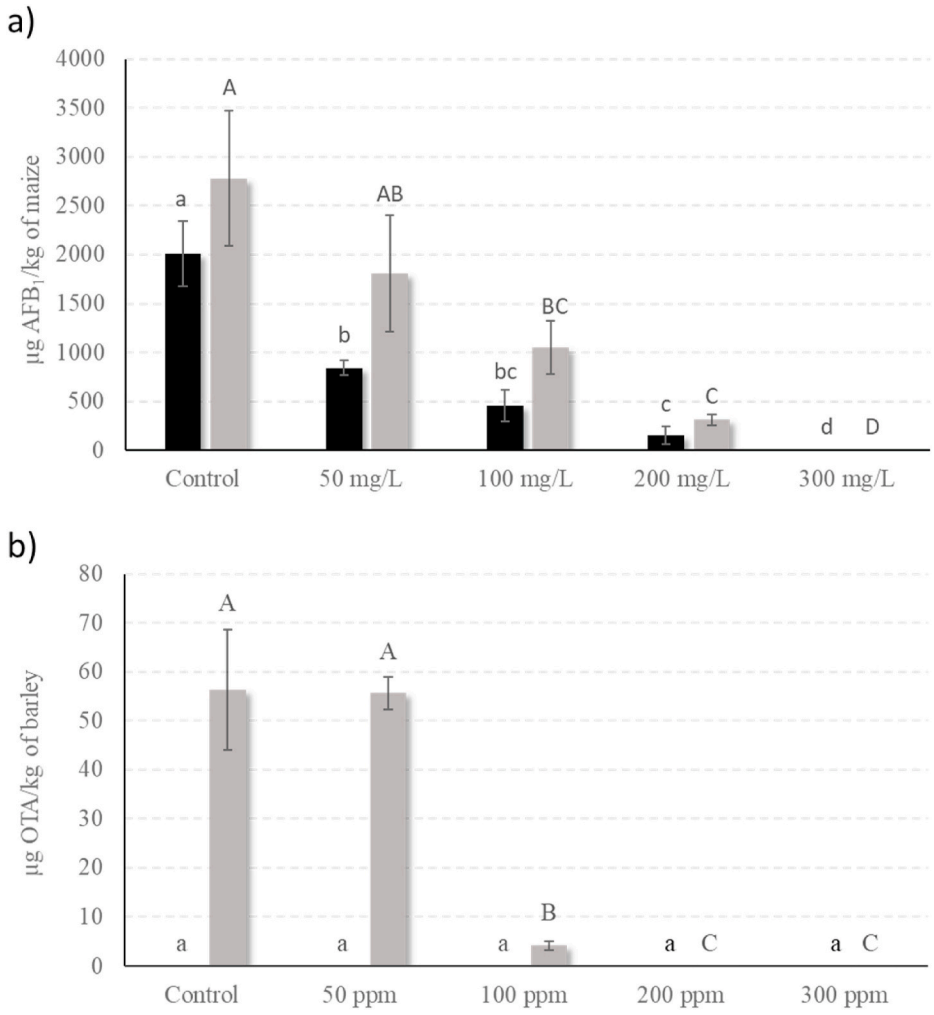

Fig. 4. AFB_1 detected in maize contaminated with *A. flavus* ITEM 8111 (a) and OTA detected in barley contaminated with *P. verrucosum* VTT D-01847 (b) in a 1L jar, trated to PA vapor by HEC gel after 1 week (dark grey) and 2 weeks (light grey) of incubation. Significant differences between treatments within each week are marked with different letters ($p \leq 0.05$).

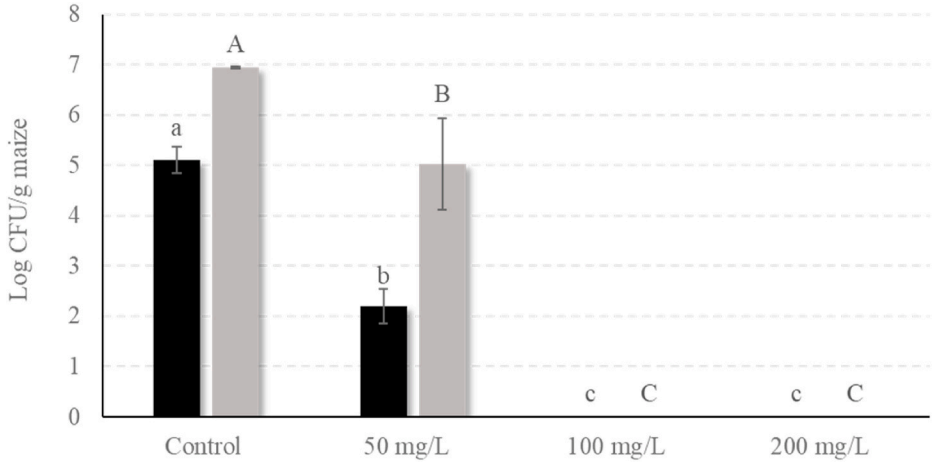

Fig. 5. Microbiological growth in uncontaminated maize in a 1L jar, trated to PA vapor by HEC gel after 1 week (dark grey) and 2 weeks (light grey) of incubation. Significant differences between treatments within each week are marked with different letters ($p \leq 0.05$).

mg/L inside the jar reduced the fungal population by 2.90 Log CFU/g (99.86%) while 100 mg/L eliminated all fungal load. In week two the results were similar, with a reduction of 1.91 Log CFU/g (97.13%) for the treatment of 50 mg/L, while 100 mg/L eliminated all fungal load.

This test proves that under conditions of natural contamination, the PA doses required to inhibit fungal growth is lower than those required in section 3.3.

4. Conclusions

The present study demonstrated the ability of PA in a volatile form to eliminate or reduce the growth of the toxigenic fungi *A. flavus* (ITEM 8111) and *P. verrusocum* (D-01847 VTT) both *in vitro* and in stored cereals (maize and barley, respectively). Likewise, PA could inhibit the synthesis of AFB_1 and OTA mycotoxins when their producing fungi were exposed to doses between 100 and 200 ppm. These concentrations are similar to those of other compounds such as EO from vegetable extracts, so it is presented as an interesting alternative to reduce fungal contamination and to extend the storage time of cereals for human consumption.

CRediT authorship contribution statement

Carlos Luz: Formal analysis, Data curation, performed the experiments and analyzed the data, wrote the paper. **Raquel Carbonell:** Formal analysis, Data curation, performed the experiments and analyzed the data. **Juan Manuel Quiles:** designed the study. **Raquel Torrijos:** Writing – original draft, Nazareth contributed to the writing of the manuscript. **Tiago de Melo Nazareth:** Writing – original draft, Nazareth contributed to the writing of the manuscript. **Jordi Mañes:** conceived and proposed the idea. **Giuseppe Meca:** conceived and proposed the idea, designed the study.

Declaration of competing interest

The authors state that there is no conflict of interest issue in relation to the manuscript above.

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References

Abass, A. B., Ndunguru, G., Mamiro, P., Alenkhe, B., Mlingi, N., & Bekunda, M. (2014). Post-harvest food losses in a maize-based farming system of semi-arid savannah area of Tanzania. *Journal of Stored Products Research, 57*, 49–57. https://doi.org/10.1016/j.jspr.2013.12.004

Alshannaq, A., & Yu, J. H. (2017). Occurrence, toxicity, and analysis of major mycotoxins in food. *International Journal of Environmental Research and Public Health, 14*(6). https://doi.org/10.3390/ijerph14060632

Banach, J. L., van Bokhorst-van de Veen, H., van Overbeek, L. S., van der Zouwen, P. S., Zwietering, M. H., & van der Fels-Klerx, H. J. (2020). Effectiveness of a peracetic acid solution on *Escherichia coli* reduction during fresh-cut lettuce processing at the laboratory and industrial scales. July 2019 *International Journal of Food Microbiology, 321*. https://doi.org/10.1016/j.ijfoodmicro.2020.108537, 108537.

* Bernardi, A. O., Stefanello, A., Garcia, M. V., Parussolo, G., Stefanello, Moro, C. B., et al. (2018). Efficacy of commercial sanitizers against fungi of concern in the food industry. *LWT, 97*, 25–30. https://doi.org/10.1016/j.lwt.2018.06.037. This reference is key because it allows us to compare our results with those obtained by official methods.

Darfour, B., & Rosentrater, K. A. (2020). Cost assessment of five different maize grain handling techniques to reduce postharvest losses from insect contamination. *Insects, 11*(1), 1–22. https://doi.org/10.3390/insects11010050

Dumont, M., Orsat, V., & Raghavan, V. (2016). Emerging technologies for promoting food security, overcoming the world food crisis. *Woodhead Publishing Series in Food Science, Technology and Nutrition*, 135–156. https://doi.org/10.1016/B978-1-78242-335-5.18001-1

EC. (2006). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1881&from=EN. (Accessed 20 December 2020).

European Standard n. 13697. (2001). *Chemical disinfectants and antiseptics – quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas – test method and requirements without mechanical action (phase 2, step 2). Main international standard for efficacy testing of commercial sanitizers*.

García-Díaz, M., Patiño, B., Vázquez, C., & Gil-Serna, J. (2019). A novel niosome-encapsulated essential oil formulation to prevent *Aspergillus flavus* growth and aflatoxin contamination of maize grains during storage. *Toxins, 11*(11). https://doi.org/10.3390/toxins11110646

García-Lara, S., García-Jaimes, E., & Ortíz-Islas, S. (2020). Field effectiveness of improved hermetic storage technologies on maize grain quality in Central Mexico. *Journal of Stored Products Research, 87*. https://doi.org/10.1016/j.jspr.2020.101585

Gitonga, Z. M., De Groote, H., Kassie, M., & Tefera, T. (2013). Impact of metal silos on households' maize storage, storage losses and food security: An application of a propensity score matching. *Food Policy, 43*, 44–55. https://doi.org/10.1016/j.foodpol.2013.08.005

Hassaballah, A. H., Bhatt, T., Nyitrai, J., Dai, N., & Sassoubre, L. (2020). Inactivation of: E. coli, Enterococcus spp., somatic coliphage, and cryptosporidium parvum in wastewater by peracetic acid (PAA), sodium hypochlorite, and combined PAA-ultraviolet disinfection. *Environmental Science: Water Research and Technology, 6*(1), 197–209. https://doi.org/10.1039/c9ew00837c

Hua, H., Xing, F., Selvaraj, J. N., Wang, Y., Zhao, Y., Zhou, L., et al. (2014). Inhibitory effect of essential oils on *Aspergillus ochraceus* growth and ochratoxin A production. *PLoS One, 9*(9). https://doi.org/10.1371/journal.pone.0108285. e108285.

Jian, F., & Jayas, D. S. (2012). The ecosystem Approach to grain storage. *Agricultural Research, 1*(2), 148–156. https://doi.org/10.1007/s40003-012-0017-7

* Kyanko, M. V., Russo, M. L., Fernández, M., & Pose, G. (2010). Effectiveness of peracetic acid on fungal spores reduction of moulds causing rotting in fruits and vegetables. *Informacion Tecnologica, 21*, 125–130. https://doi.org/10.4067/S0718-07642010000400016. This reference is key because it uses a target fungus of the same species and genus as ours (A. flavus).

Leggett, M. J., Spencer Schwarz, J., Burke, P. A., Mcdonnell, G., Denyer, S. P., & Maillard, J. Y. (2015). Resistance to and killing by the sporicidal microbicide peracetic acid. *Journal of Antimicrobial Chemotherapy, 70*(3), 773–779. https://doi.org/10.1093/jac/dku445

Nazareth, T. M., Bordin, K., Manyes, L., Meca, G., Mañes, J., & Luciano, F. B. (2016). Gaseous allyl isothiocyanate to inhibit the production of aflatoxins, beauvericin and enniatins by *Aspergillus parasiticus* and *Fusarium poae* in wheat flour. *Food Control, 62*, 317–321. https://doi.org/10.1016/j.foodcont.2015.11.003

Nazareth, T. de M., Quiles, J. M., Torrijos, R., Luciano, F. B., Mañes, J., & Meca, G. (2019). Antifungal and antimycotoxigenic activity of allyl isothiocyanate on barley under different storage conditions. *Lebensmittel-Wissenschaft & Technologie, 112* (June). https://doi.org/10.1016/j.lwt.2019.06.004, 108237.

Nicolau-Lapeña, I., Abadias, M., Bobo, G., Aguiló-Aguayo, I., Lafarga, T., & Viñas, I. (2019). Strawberry sanitization by peracetic acid washing and its effect on fruit quality. *Food Microbiology, 83*(April), 159–166. https://doi.org/10.1016/j.fm.2019.05.004

* Ocak, I., Çelik, A., Özel, M. Z., Korcan, E., & Konuk, M. (2012). Antifungal activity and chemical composition of essential oil of *origanum hypericifolium*. *International Journal of Food Properties, 15*(1), 38–48. https://doi.org/10.1080/10942911003687249. This reference is key because it analyzes the effect of AP against many fungi of the genera Aspergillus and Penicillium.

OECD/FAO. (2020). *OECD-FAO Agricultural Outlook 2020-2029*. Paris: FAO, Rome/OECD Publishing. https://doi.org/10.1787/1112c23b-en

* Olivier, B. A., Stefanello, A., Goncalves, L. J., Valle, G. M., & Venturini, C. M. (2019). Antifungal activity of commercial sanitizers against strains of Penicillium roqueforti, Penicillium paneum, Hyphopichia burtonii, and Aspergillus pseudoglaucus: Bakery spoilage fungi. Food Microbiology 83: 59-63. https://doi.org/10.1016/j.fm.2019.04.005 This reference is key because it analyzes the effect of AP against fungi contaminating food products.

OMRI. (2016). *Organic materials review Institute. Technical evaluation Report, peracetic acid crops*. USA: USDA National Organic Program.

Paris, M. J., Ramírez-Corona, N., Palou, E., & López-Malo, A. (2020). Modelling release mechanisms of cinnamon (*Cinnamomum zeylanicum*) essential oil encapsulated in alginate beads during vapor-phase application. *Journal of Food Engineering, 282*. https://doi.org/10.1016/j.jfoodeng.2020.110024, 110024.

* Quiles, J. M., Nazareth, T. M., Luz, C., Luciano, F. B., Mañes, J., & Meca, G. (2019). Development of an antifungal and antimycotoxigenic device containing allyl isothiocyanate for silo fumigation. *Toxins, 11*(3), 137. https://doi.org/10.3390/toxins11030137. This reference is key because of the use of the same type of compound (HC) for the formulation of the antifungal gels.

Rao, S., Xu, G., Lu, X., Zhang, R., Gao, L., Wang, Q., et al. (2020). Characterization of ovalbumin-carvacrol inclusion complexes as delivery systems with antibacterial application. *Food Hydrocolloids, 105*. https://doi.org/10.1016/j.foodhyd.2020.105753, 105753.

Rasooli, I., Fakoor, M. H., Yadegarinia, D., Gachkar, L., Allameh, A., & Rezaei, M. B. (2008). Antimycotoxigenic characteristics of *Rosmarinus officinalis* and *Trachyspermum copticum L.* essential oils. *International Journal of Food Microbiology, 122*, 135–139. https://doi:10.1016/j.ijfoodmicro.2007.11.048.

Rasooli, I., & Owlia, P. (2005). Chemoprevention by thyme oils of *Aspergillus parasiticus* growth and aflatoxin production. *Phytochemistry, 66*, 2851–2856. https://doi.org/10.1016/j.phytochem.2005.09.029

Serrano, A. B., Font, G., Mañes, J., & Ferrer, E. (2013). Emerging Fusarium mycotoxins in organic and conventional pasta collected in Spain. *Food and Chemical Toxicology, 51*, 259e266 https://doi:10.1016/j.fct.2012.09.034.

Siah, A., Deweer, C., Morand, E., Reignault, P., & Halama, P. (2010). Azoxystrobin resistance of French *Mycosphaerella graminicola* strains assessed by four in vitro bioassays and by screening of G143A substitution. *Crop Protection, 29*, 737–743. https://doi.org/10.1016/j.cropro.2010.02.012

Singano, C. D., Mvumi, B. M., & Stathers, T. E. (2019). Effectiveness of grain storage facilities and protectants in controlling stored-maize insect pests in a climate-risk

prone area of Shire Valley, Southern Malawi. *Journal of Stored Products Research, 83*, 130–147. https://doi.org/10.1016/j.jspr.2019.06.007

Ul Hassan, Z., Al Thani, R., Alnaimi, H., Migheli, Q., & Jaoua, S. (2019). Investigation and application of *Bacillus licheniformis* volatile compounds for the biological control of toxigenic *Aspergillus* and *Penicillium spp*. *ACS Omega, 4*(17), 17186–17193. https://doi.org/10.1021/acsomega.9b01638

Wang, S., Liu, H., Lin, J., & Cao, Y. (2010). Can ozone fumigation effectively reduce aflatoxin B1 and other mycotoxins contamination on stored grain?. In *10th International working Conference on stored product Protection*. https://doi:10.5073/jka.2010.425.167.172.

Wang, D., Yamaki, S., Kawai, Y., & Yamazaki, K. (2020). Sanitizing efficacy and antimicrobial mechanism of peracetic acid against histamine-producing bacterium, Morganella psychrotolerans. *Lebensmittel-Wissenschaft & Technologie, 126*(March). https://doi.org/10.1016/j.lwt.2020.109263, 109263.

Yu, X., Luo, H., Wang, H., & Feil, J. H. (2020). Climate change and agricultural trade in central Asia: Evidence from Kazakhstan. *Ecosystem Health and Sustainability, 6*(1). https://doi.org/10.1080/20964129.2020.1766380