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Comparative Antimycotic Effects of Selected Herbs, Spices, Plant Components and Commercial Antifungal Agents[1]

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ABSTRACT

The antifungal effects of 16 ground herbs and spices, 4 other plant materials, 3 commercial antifungal agents, tannic acid and 2 experimental mold inhibitors were tested against seven mycotoxin-producing molds. Of the 26 substances tested, cloves, cinnamon, mustard, allspice, garlic, and oregano at the 2% level in potato dextrose agar, completely inhibited growth of all seven mycotoxigenic molds for various times up to 21 d. The remaining compounds either caused little or no inhibition. Powdered pomegranate peel was a good inhibitor against four *Penicillium* species. Potassium sorbate at 0.3% was highly effective against all seven mold strains. The antifungal antibiotic, natamycin (pimaricin), was also highly effective. Combinations of different levels of potassium sorbate and cloves showed an enhanced or possible synergistic inhibitory effect on growth of all seven molds tested, indicating the possibility of using spices and commercial antifungal agents together in small amounts to obtain antimycotic activity.

Herbs and spices are widely used to impart flavor to foods. Usage of herbs and spices in the U.S. has increased considerably to the extent that now more than 86,000 tons (7.8×10^7 kg) are imported annually (*12*). While it is generally accepted that certain herbs and spices have antimicrobial activities and may influence the keeping quality of foods to which they have been added, they are currently not used with the primary purpose of exerting a preservative effect.

The preservative action of herbs and spices has only recently received attention in the literature where studies (*1,7,8,13,14,15,17,18,19*) have been reported and show that mycotoxin-producing molds may be inhibited by some herbs and spices. The study reported here deals with the inhibitory effects of 16 different commercial herbs and spices, 4 plant products, tannic acid and 5 commercial or potential antimycotic agents on the growth of seven known toxigenic mold strains. The antimycotic agents were included for comparative purposes.

MATERIALS AND METHODS

Substances tested

Sixteen herbs and spices were chosen for this study on the basis of their reported antimicrobial activity or from the results obtained from preliminary studies. The herbs and spices used were as follows: ground cloves (*Eugenia caryophyllus*), cinnamon (*Cinnamomum zeylanicum* B.) mustard (*Sinapis alba* L.), allspice (*Pimenta*), oregano (*Oreganum*), thyme (*Thymus*), turmeric (*Curcuma longa* L.), anise (*Pimpinella onisum*), paprika (*Capsicum annum*), red pepper (*Red Cayenne*), black pepper (*Piper nigrum* L.), white pepper (*Piper white*), leaves of sage (*Salvia*) and rosemary (*Rossmarinus*), onion (*Allium cepa* L.) and garlic (*Allium satiuum*) powders. Four other plant-derived substances, namely, dried carrot leaves, dried green tea leaves, dried pomegranate peels, dried orange peels and tannic acid, were also studied. All spices, herbs and plant-derived substances were purchased from a local supermarket and tannic acid was obtained from J. W. Maranville, Sorghum Laboratory, University of Nebraska. Five antifungal compounds were also used in this study to compare their effects with the other substances; these were: natamycin (Gist-Brocades N.V., Delft, Holland), lauricidin (donated by J. J. Kabara, Department of Biomechanics, Michigan State University, East Lansing, MI), potassium sorbate and sodium benzoate (Monsanto Industrial Chemicals Co., St. Louis, MO) and calcium propionate (Pfizer Inc., Chemicals Division, New York, NY).

Test organisms

Seven strains of mycotoxigenic molds were used as test organisms, including: *Aspergillus flavus* NRRL A16900 and *Aspergillus parasiticus* NRRL 2999 (aflatoxins), *Aspergillus ochraceus* NRRL 3174 (ochratoxin), *Penicillium* species M46 (ochratoxin), *Penicillium patulum* M59 (patulin), *Penicillium roquefortii* M247 (patulin) and *Penicillium citrinum* M565 (citrinin). The *Aspergillus* species were obtained from the Culture Collection of the USDA Northern Regional Research Center, ARS, Peoria, ILL, and the *Penicillium* species were all from our culture collection and had previously been isolated from cheese. All cultures were maintained on potato dextrose agar (PDA) slants.

Inoculum

Spores were from 7-10-d-old PDA slant cultures of the organisms grown at ca. 25°C were used to inoculate medicine bottles containing PDA. These cultures were incubated for 7-10 d at ca. 25°C until well sporulated. Then 50 ml of sterile 0.05% Tween-80 were added, and the spores were loosened using sterile glass beads. Each suspension was filtered through cheesecloth to remove mycelial fragments. The spore suspensions were adjusted with sterile 0.05% Tween-80 to contain approxi-

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TABLE 1. *Days[a] for initiation of mold growth on potato dextrose agar containing different spices at 2% (w/v).*

Organisms	Spices								
	Control	Cloves	Cinnamon	Mustard	Garlic	Allspice	Oregano	White pepper	Black pepper
A. flavus	2	>21	7	7	6	4	4	3	3
A. parasiticus	2	>21	8	7	7	6	4	4	3
A. ochraceus	2	>21	10	7	6	5	4	2	3
Penicillium sp. M46	2	>21	>21	7	10	7	6	3	2
P. roqueforti	2	>21	7	7	6	6	9	2	2
P. patulum	2	>21	>21	6	5	6	9	2	2
P. citrinum	2	>21	>21	9	6	>21	9	3	2

[a]Average of triplicate values.

mately 10^6 spores/ml based on total counts obtained by both surface plating on PDA and using Petroff-Hauser Counting Chamber.

Preparation of PDA-spice plates and inoculation

One half of circular bi-divided petri plates was filled with PDA as a control, then 18 ml of PDA in 125-ml Erlenmeyer flasks were sterilized by autoclaving at 121°C for 15 min; immediately after removal of the agar from the autoclave, 2% spice (w/v) was added to each flask. This helped reduce contaminants, particularly mold spores and vegetative bacteria. The agar/spice mixture was poured into the other half of the petri plates until the upper surface was even with the control, forming a continuous surface. The same procedure was used for the antifungal compounds and tannic acid. Sorbate, benzoate, propionate, lauricidin and tannic acid were used at a level of 0.3% (w/v) and natamycin was used at 5 μg/ml (ppm). Combination of 0.1% cloves with 0.1, 0.2 or 0.3% sorbate were also employed. The total surface of each plate was inoculated with 0.1 ml of the spore suspension (ca. 10^5 spores) and evenly spread over the entire surface using a flamed bent glass rod. The plates were inverted and incubated at ca. 25°C. Mold growth was observed visually throughout the incubation period for 21 d, and the times for initiation of mold growth on the PDA control and the PDA plus the different substances were recorded.

RESULTS AND DISCUSSION

From data in Table 1, it can be seen that clove was the strongest antifungal spice; it inhibited growth of all seven mold strains for more than 21 d. Cinnamon was also quite inhibitory, and mustard, garlic, allspice and oregano gave various degrees of inhibition. With mustard, the initiation of mold growth on the PDA control side was also delayed for about 6 d when usually only 2 d were required to initiate growth by the control. This delay in growth appeared to be due to strong volatile compounds from mustard, which also affected the control side. Mustard was also a very effective inhibitor until about 7 to 9 d, when mold growth became evident. This again appeared to be due to the volatility of the inhibitory compounds, since once the compound had volatilized, mold growth rapidly developed.

The other spices, white pepper, black pepper, rosemary, tyhyme, tumeric, anise, and onion had relatively minor effects on growth of the *Apsergillus* and *Penicillium* strains, while sage, red pepper, and paprika had no effect under conditions of our study.

Hoffman and Evans (*16*) were among the earliest to describe the preservative action of cinnamon, cloves, mustard, allspice, nutmeg, ginger, black pepper, and cayenne pepper. They found that cinnamon, cloves, and mustard were the most effective and ginger, black pepper, and cayenne pepper were the least effective.

Bachmann (*4,5*) studied the effect of spices and their essential oils on growth of several test organisms, including *Aspergillus* and *Penicillium* species, and concluded that spices used in amounts as employed normally for ordinary foods were insufficient as preservatives. However, when used in larger amounts, cinnamon, cloves, and allspice retarded mold growth. Bullerman (*7*) reported that cinnamon in concentrations as low as 0.02% inhibited mold growth and aflatoxin production in yeast extract sucrose (YES) broth. Hitokoto et al. (*14*) reported that cloves and allspice completely inhibited growth of *A. flavus, A. parasiticus* and *A. ochraceus*. Sage leaves caused partial inhibition of growth of *A. ochraceus;* turmeric and anise showed complete inhibition of *A. ochraceus* and partial inhibition of *A. flavus*. Rosemary, paprika and pepper seeds had relatively minor effects on all three *Aspergillus* species. Llewellyn et al. (*17*) reported that clove, cinnamon, thyme and oregano had antimycotic or antitoxigenic effects on aflatoxin producing molds. The data listed in Table 1 are only in partial agreement with those published by Hitokoto et al. (*14*) and Llewellyn et al. (*17*). Agreement was found in cinnamon, cloves, oregano, and allspice, which completely or partially inhibited growth of test organisms. However, data from our study do not support their reports that thyme, anise, sage and turmeric caused either complete or partial inhibition of some strains. According to our results, these compounds had little effect on the mold strains used in this study. This discrepancy may be due to the different mold strains and levels of herbs and spices used. Our experience with these compounds indicated that they are used in the food industry in levels up to 2% and our levels of testing were set accordingly. Hitokoto et al. (*14*), on the other hand, used approximately 11.1%, and Llewellyn et al. (*17*) grew their molds directly on moistened spices.

Appleton and Tansey (*2*) reported that 0.1% of an aqueous extract of garlic bulbs inhibited growth of many species of zoopathogenic fungi. Our results also showed that garlic powder inhibited all seven mold strains for several days but the effect was not indefinite. Since we used powdered garlic, we may have observed a lessened effect due to possible loss of a volatile active ingredient during drying.

Dold and Knapp (*11*) reported that spices which contain tannins or alkaloids were among the most germicidal. This

TABLE 2. *Days[a] for initiation of mold growth on potato dextrose agar containing antifungal compounds.*

Organisms	Control	Compounds[b] K-sorbate	Na-benzoate	Ca-propionate	Lauricidin	Natamycin
A. flavus	2	6	3	2	4	7
A. parasiticus	2	4	3	2	2	7
A. ochraceus	2	18	3	3	2	>21
Penicillium sp. M46	2	6	3	2	3	>21
P. roqueforti	2	5	3	2	3	>21
P. patulum	2	6	3	2	2	>21
P. citrinum	2	18	5	3	3	>21

[a]Average of triplicate values.
[b]Natamycin 5 μg/ml; all others 0.3%.

TABLE 3. *Days[a] for initiation of mold growth on potato dextrose agar containing cloves, different levels of sorbate and combinations of cloves and sorbate.*

Organisms	Control	Cloves (0.1%)	Sorbate 0.1%	0.2%	0.3%	Cloves (0.1%) plus sorbate 0.1%	0.2%	0.3%
A. flavus	2	3	4	5	6	7	8	9
A. parasiticus	2	3	3	3	4	6	6	8
A. ochraceus	2	4	9	12	18	9	15	>21
Penicillium sp. M46	2	5	4	5	6	7	8	12
P. roqueforti	2	3	3	4	5	6	8	9
P. patulum	2	4	4	5	6	7	8	10
P. citrinum	2	5	9	12	18	9	15	>21

[a]Average of triplicate values.

suggested the testing of tea and pomegranate peels, since tea contains tannins and alkaloids and pomegranate peels are high in tannins (*6*). Pomegranate peels (data not shown) inhibited growth of *P. citrinum* for 8 d, *Penicillium* sp. M46 for 6 d, *P, patulum* for 4 d and *A. ochraceus* and *P. roquefortii* for 3 d. However, pomegranate peels had no effect on *A. flavus* or *A. parasiticus*. Green tea leaves inhibited all four *Penicillium* strains for 3 d, but had no effect on the *Aspergillus* species. Carrot leaves and orange peels had no inhibitory effect on mold growth. The testing of orange peels was suggested by a study by Alderman and Marth(*1*), who reported that citrus essential oils inhibited and growth of *A. parasiticus*. However, our study showed that dried orange peels had no effect on any of the mold strains tested. Again, the level used and volatility of the active ingredient may have affected the inhibitory activity. Condon and Kuc (*10*) reported isolation of a fungitoxic compound from carrots. Later, Batt et al. (*3*) reported that carrot root extract inhibited *A. parasiticus*. We found that dried carrot leaves had no inhibitory effect on growth of the molds tested.

Table 2 lists the time for initiation of growth on potassium sorbate, sodium benzoate, calcium propionate, tannic acid and lauricidin at 0.3%, and natamycin at 5 ppm. Potassium sorbate inhibited growth of *A. ochraceus* and *P. citrinum* for 18 d; *A. parasiticus* for 4 d and *P. roqueforti* for 5 d. Sodium benzoate was less effective in inhibiting molds than potassium sorbate, but it was more effective than calcium propionate. Tannic acid and lauricidin were either slightly effective or had no effect. Natamycin inhibited *A. flavus* and *A. parasiticus* for 7 d and the rest for 21 d or longer.

Of the three commercial antifungal compounds tested, potassium sorbate was most effective. Sodium benzoate was more effective against all mold strains employed than calcium propionate. According to Chipley and Uraih (*9*), derivatives of benzoic acid, such as methyl benzoate and ethyl benzoate, were effective in reducing both mycelial growth and aflatoxin production by *A. flavus* and *A. parasiticus*.

Natamycin which is produced by *Streptomyces natalinsis* is a strong antifungal agent effective at very low levels (5 ppm). It was included in this study for compartive purposes and because of possible future applications as a food preservative in the U.S.

Table 3 lists the time for initiation of mold growth on different levels of sorbate in combination with 0.1% cloves. Sorbate and clove had a combined inhibitory effect. The effect was certainly additive in all instances, and in some instances, may have been synergistic.

It is of interest that combining clove and potassium sorbate produced a more pronounced effect in inhibiting growth of the mold strains. This effect in inhibiting mold growth indicates the possibility of combining spices and commercial antifungal compounds in small quantities for use as antifungal agents. However, further studies of the combined effects of spices and commercial antifungal compounds should be done to evaluate the practical significance.

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