

## Influence of pH Alone and in Combination with Phenolic Antioxidants on Growth and Germination of Mycotoxigenic Species of *Fusarium* and *Penicillium*

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### ABSTRACT

The effect of pH alone and in combination with two phenolic antioxidants on mycelial growth and conidial germination of mycotoxigenic fungi was examined. The mycotoxigenic fungi studied included seven members each of *Fusarium* and *Penicillium* genera. The present study was carried out at pH 4, 6, 8, and 10 alone and in the presence of 100 and 200 µg/ml of butylated hydroxyanisole (BHA) and propyl paraben, respectively. All of the test fungi examined were able to grow at pH 4, 6, 8, and 10 on potato dextrose agar. The majority of the *Fusarium* species, with the exception of *Fusarium graminearum*, were tolerant of acid and alkaline pHs. Two of the *Penicillium* species were rapid growers and were tolerant of acid and alkaline pHs. The tolerance of the slower growing *Penicillium* species varied in their response to acid and alkaline pHs. Conidial germination was partially inhibited in all of the *Fusarium* and *Penicillium* species in the presence of 100 µg/ml of BHA at all pH levels. However, in the presence of 200 µg/ml of BHA, there was complete inhibition in conidial germination for four of the *Fusarium* species at pH 4, 6, 8, and 10. Propyl paraben, at the four pH levels, completely inhibited conidial germination in *Fusarium* species and all but one species of *Penicillium* at 200 µg/ml. Reduction in colony diameter for the majority of *Fusarium* and *Penicillium* species, at all pH levels was observed at 100 and 200 µg/ml of BHA. However, at 200 µg/ml of Propyl paraben, mycelial growth was completely inhibited for the majority of the *Fusarium* and *Penicillium* species at the four pH levels.

The fungitoxic effect of phenolic antioxidants on conidial germination, mycelial growth, and aflatoxin production has been studied extensively (1,2,6-8,10,13). Chang and Branen (1) reported that 1,000 ppm of butylated hydroxyanisole (BHA) totally prevented growth and aflatoxin production of *Aspergillus parasiticus* spores, while 250 ppm or greater inhibited mycelium growth of *A. parasiticus*. In another study by Lin and Fung (10), the minimum inhibitory concentration of BHA and tertiary butylhydroquinone was reported to be 0.0005 and 0.001 g per plate, respectively. In 1991, Thompson (13) reported that 200 µg/ml and above prevented conidial germination in two species of *Aspergillus*. These studies were important because the species of *Aspergillus* used were aflatoxin

producers. However, other genera of fungi such as *Fusarium* and *Penicillium* include species that also produce toxins. But, limited information is available concerning the effect of antioxidants on the growth and toxin production of the toxigenic fungi. Thus, the objective of this investigation were to determine the effect of two antioxidants, 2(3)-tert-butyl-4-hydroxyanisole (butylated hydroxyanisole) (BHA) and propyl ester of p-hydroxybenzoic acid (propyl paraben) (PP) on mycelial growth and conidial germination of known mycotoxin-producing species of *Fusarium* and *Penicillium* and to determine the effect of pH on the fungitoxic activity of BHA and PP.

### MATERIALS AND METHODS

#### Stock cultures

Mycotoxin-producing species of *Fusarium* and *Penicillium* were purchased from the American Type Culture Collection (Rockville, MD). The *Fusarium* species considered to be important from a public health viewpoint were *Fusarium graminearum* ATCC 56749, which produces moniliformin, *Fusarium moniliforme* ATCC 38946 (moniliformin), *Fusarium oxysporum* ATCC 46995 (zearalenone), *Fusarium poae* ATCC 60317 (diacetoxyscirpenol), *Fusarium roseum* ATCC 28114 (zearalenone, deoxynivalenol, acetyl-deoxynivalenol, and vomitoxin), and *Fusarium sporotrichioides* ATCC 24630 (T-2 toxin, HT-2 toxin, butenolide, and neosolaniol). The toxigenic *Penicillium* species were *Penicillium aurantiogriseum* ATCC 58608 (which produces penicillic acid and viridicatin), *Penicillium aurantiogriseum* ATCC 58604 (penicillic acid, viomellein, viridicatin, and xanthomegnin), *Penicillium camembertii* ATCC 58603 (cyclopiazonic acid and rugulovasine A and B), *Penicillium chrysogenum* ATCC 58611 (penicillin, PR-toxin, and roquefortine), *Penicillium griseofulvum* ATCC 11885 (griseofulvin), *Penicillium purpurogenum* ATCC 20204 (rugulovasine A and B), and *Penicillium purpurogenum* ATCC 26940 (rubratoxin). Cultures were maintained at 5°C on slants of potato dextrose agar (PDA).

#### Culture medium

PDA was adjusted to pH 4, 6, 8, and 10 using 5 M H<sub>3</sub>PO<sub>4</sub> for pH values 4 and 6 and 1 M NaOH for pH values 8 and 10. Actual pH values were determined before and after growth with a general purpose Ag/AgCl refillable pH electrode attached to a pH meter.

The medium was amended with the appropriate amount of phenolic antioxidant. The final phenolic antioxidant concentration in the culture medium was 0, 100, 200, and 300 µg/ml before autoclaving.

#### Chemical

BHA and PP were purchased from Fluka Chemical Company (Ronkonkoma, New York). Stock solutions of the phenolic antioxidant were made in 95% ethyl alcohol.

#### Spore germination determinations

For the experiments on conidial germination, germination was defined as the extension of a germ tube to a length equal to one-half the diameter of the conidium (11). Germination was reported as a percentage of the conidial population, determined by microscopic count, and evaluated on solid agar medium (5 ml PDA per plate) in standard size petri plates. At least 400 conidia were counted for each sample.

A 7-d culture of each test fungus grown on PDA slants was flooded with 5 ml of sterile, distilled water to obtain a conidial suspension. The conidial suspension was passed through sterile cheese cloth and centrifuged (10,000 g, min) in three changes of sterile distilled water. A one-tenth ml conidial inoculum (ca.  $5 \times 10^6$  conidia) was spread over the surface of each PDA plate (amended with the appropriate concentration of antioxidants at pH 4, 6, 8, and 10) with an alcohol-flamed glass rod. The plates were allowed to incubate for 24 h at 27°C. After the incubation period, agar blocks (5 mm x 5 mm) were aseptically removed with a scalpel (5 mm x 10 mm). The blocks were placed on clean microscope slides under cover slips and examined under 100 and 400X magnification. For each sample, at least 400 conidia were counted. Microscopic fields were selected randomly. The germination percentage was determined and compared with an appropriate control receiving a similar volume of 95% ethanol. Three replicate plates per treatment were used and the experiments were repeated twice.

#### Colony diameter determinations

PDA, amended with the appropriate antioxidant at pH 4, 6, 8, and 10, was spot inoculated with a loop of a conidial suspension (ca.  $5 \times 10^6$ ) of each fungus studied (4). The inoculated plates were incubated for 7 d at 27°C. At the end of the incubation period, the colony diameter was measured. For each colony two diameters, measured at right angles to one another, were averaged to find the mean diameter for that colony. Inhibition was measured as the percent decrease in colony diameters compared with the colony diameters in the control medium (PDA) in the absence of antioxidant. The percentage of reduction was calculated as follows: % reduction =  $100 - (\text{diameter on medium with antioxidant} / \text{diameter on PDA})$ . All colony diameters were determined by using two replicates for each test fungus and the experiment was repeated two times.

All experimental data were analyzed statistically using the Duncan's new multiple range test ( $P = 0.05$ ).

## RESULTS

Mycelial growth of *Fusarium* and *Penicillium* species at four pH levels is shown in Table 1. Similar growth patterns were observed for the majority of *Fusarium* species at pH 6 and 8. *F. poae* and *F. sporotrichioides* exhibited identical growth patterns at all pH levels, while *F. moniliforme* and *F. oxysporum* also exhibited identical growth patterns.

There was wide variation in the mycelial growth of *Penicillium* species at the four pH levels. Both strains of *P.*

TABLE 1. The effect of pH on mycelial growth of *Fusarium* and *Penicillium* species grown on PDA for 7d at 27°C.

Fungi	Radial growth (mm)*			
	pH 4	6	8	10
<i>F. graminearum</i> Schwabe ATCC 56749	60 <sup>b</sup>	80 <sup>a</sup>	40 <sup>c</sup>	25 <sup>d</sup>
<i>F. moniliforme</i> Sheldon ATCC 38946	70 <sup>b</sup>	80 <sup>a</sup>	80 <sup>a</sup>	80 <sup>a</sup>
<i>F. oxysporum</i> Schlechtendahl ATCC 46995	70 <sup>b</sup>	80 <sup>a</sup>	80 <sup>a</sup>	80 <sup>a</sup>
<i>F. poae</i> (Peck) Wollenweber ATCC 60317	80 <sup>a</sup>	80 <sup>a</sup>	80 <sup>a</sup>	80 <sup>a</sup>
<i>F. roseum</i> Link ATCC 28113	70 <sup>b</sup>	80 <sup>a</sup>	80 <sup>a</sup>	75 <sup>a</sup>
<i>F. sporotrichioides</i> Sherbackoff ATCC 24630	80 <sup>a</sup>	80 <sup>a</sup>	80 <sup>a</sup>	80 <sup>a</sup>
<i>P. aurantiogriseum</i> Dierckx ATCC 58603	51 <sup>a</sup>	30 <sup>b</sup>	35 <sup>b</sup>	25 <sup>c</sup>
<i>P. aurantiogriseum</i> Dierckx ATCC 58604	25 <sup>b</sup>	27 <sup>b</sup>	40 <sup>a</sup>	38 <sup>a</sup>
<i>P. camembertii</i> Thom ATCC 58608	12 <sup>c</sup>	20 <sup>b</sup>	30 <sup>a</sup>	20 <sup>b</sup>
<i>P. chrysogenum</i> Thom ATCC 58611	35 <sup>b</sup>	45 <sup>a</sup>	40 <sup>a</sup>	35 <sup>b</sup>
<i>P. griseofulvum</i> Dierckx ATCC 11885	30 <sup>a</sup>	15 <sup>c</sup>	30 <sup>a</sup>	25 <sup>b</sup>
<i>P. purpurogenum</i> Stoll ATCC 20204	65 <sup>b</sup>	75 <sup>a</sup>	75 <sup>a</sup>	70 <sup>a</sup>
<i>P. purpurogenum</i> Stoll ATCC 26940	80 <sup>a</sup>	75 <sup>a</sup>	75 <sup>a</sup>	70 <sup>b</sup>

\*Values for each test fungus followed by the same letter in horizontal rows are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

*purpurogenum* grew rapidly at all of the pH levels. The optimum pH for mycelial growth was as follow: pH 4 for *P. aurantiogriseum* ATCC 58603 and *P. purpurogenum* ATCC 26940, pH 6 for *P. chrysogenum*, pH 6 and 8 for *P. purpurogenum* ATCC 20203, pH 4 and 8 for *P. griseofulvum*, and pH 8 for *P. camembertii*.

The effect of BHA and PP on conidial germination for toxigenic species of *Fusarium* and *Penicillium* at four pH levels is shown in Table 2. When compared to percent conidial germination on unamended PDA medium, there were significant reductions in percent germination of all the test fungi in the varying concentration of BHA and PP at all pH levels.

In the presence of 100 µg/ml of BHA, conidial germination was not observed at pH 10 for *F. graminearum*. The lowest percentage of conidial germination at pH 4 was recorded for *F. graminearum*, *F. poae*, and *F. sporotrichioides*, and at pH 10 for *F. moniliforme*, *F. oxysporum*, and *F. sporotrichioides*. When the concentration of BHA was increased to 200 µg/ml, further reduction in conidial germination for some species of *Fusarium* was observed and complete inhibition of conidial germination for other species at the pH levels. The lowest percentage of conidial germination at 200 µg/ml of BHA was pH 4, 6, and 8 for *F. graminearum* and pH 10 for *F. moniliforme* and *F. oxysporum*.

TABLE 2. Percentage of *Fusarium* and *Penicillium* conidia germinated when exposed to varying concentrations of BHA and PP in potato dextrose agar at pH 4, 6, 8, and 10 at 27°C.

Fungi	% Germination (24 h)*																			
	0			BHA/100			BHA/200			PP/100			PP/200							
	4	6	8	10	4	6	8	10	4	6	8	10	4	6	8	10	4	6	8	10
<i>F. graminearum</i> Schwabe ATCC 56749	80 <sup>a</sup>	85 <sup>a</sup>	60 <sup>b</sup>	59 <sup>b</sup>	15 <sup>c</sup>	40 <sup>d</sup>	50 <sup>c</sup>	0 <sup>g</sup>	5 <sup>f</sup>	4 <sup>f</sup>	4 <sup>f</sup>	0 <sup>g</sup>	5 <sup>f</sup>	10 <sup>e</sup>	10 <sup>c</sup>	8 <sup>f</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>
<i>F. moniliforme</i> Sheldon ATCC 38946	90 <sup>a</sup>	92 <sup>a</sup>	90 <sup>a</sup>	90 <sup>a</sup>	35 <sup>c</sup>	40 <sup>c</sup>	50 <sup>b</sup>	30 <sup>d</sup>	10 <sup>f</sup>	25 <sup>d</sup>	30 <sup>d</sup>	3 <sup>g</sup>	3 <sup>g</sup>	9 <sup>f</sup>	10 <sup>f</sup>	11 <sup>f</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>
<i>F. oxysporum</i> Schlechtendahl ATCC 46995	87 <sup>a</sup>	92 <sup>a</sup>	88 <sup>a</sup>	90 <sup>a</sup>	37 <sup>c</sup>	60 <sup>b</sup>	40 <sup>c</sup>	30 <sup>d</sup>	10 <sup>f</sup>	11 <sup>f</sup>	21 <sup>e</sup>	2 <sup>g</sup>	2 <sup>g</sup>	14 <sup>f</sup>	10 <sup>f</sup>	11 <sup>f</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>
<i>F. poae</i> (Peck) Wollenweber ATCC 60317	80 <sup>b</sup>	92 <sup>a</sup>	90 <sup>a</sup>	90 <sup>a</sup>	10 <sup>d</sup>	45 <sup>c</sup>	50 <sup>c</sup>	45 <sup>c</sup>	0 <sup>f</sup>	0 <sup>f</sup>	0 <sup>f</sup>	0 <sup>f</sup>	2	10 <sup>d</sup>	14 <sup>d</sup>	8 <sup>e</sup>	0 <sup>f</sup>	0 <sup>f</sup>	0 <sup>f</sup>	0 <sup>f</sup>
<i>F. roseum</i> Link ATCC 28114	85 <sup>b</sup>	92 <sup>a</sup>	90 <sup>a</sup>	90 <sup>a</sup>	10 <sup>c</sup>	25 <sup>b</sup>	40 <sup>c</sup>	2 <sup>f</sup>	0 <sup>f</sup>	0 <sup>f</sup>	0 <sup>f</sup>	0 <sup>f</sup>	0 <sup>f</sup>	9 <sup>e</sup>	10 <sup>e</sup>	0 <sup>f</sup>	0 <sup>f</sup>	0 <sup>f</sup>	0 <sup>f</sup>	0 <sup>f</sup>
<i>F. sporotrichioides</i> Sherbakoff ATCC 24630	85 <sup>b</sup>	92 <sup>a</sup>	92 <sup>a</sup>	90 <sup>a</sup>	20 <sup>c</sup>	45 <sup>c</sup>	40 <sup>c</sup>	30 <sup>d</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>	9 <sup>f</sup>	18 <sup>c</sup>	14 <sup>c</sup>	9 <sup>f</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>
<i>P. aurantiogriseum</i> Dierckx ATCC 58603	89 <sup>a</sup>	72 <sup>c</sup>	75 <sup>b</sup>	60 <sup>d</sup>	50 <sup>c</sup>	35 <sup>f</sup>	40 <sup>f</sup>	25 <sup>g</sup>	5 <sup>h</sup>	2 <sup>i</sup>	6 <sup>b</sup>	10 <sup>h</sup>	79 <sup>b</sup>	70 <sup>c</sup>	55 <sup>d</sup>	55 <sup>d</sup>	25 <sup>g</sup>	60 <sup>d</sup>	45 <sup>e</sup>	0 <sup>j</sup>
<i>P. aurantiogriseum</i> Dierckx ATCC 58604	82 <sup>a</sup>	84 <sup>a</sup>	82 <sup>a</sup>	75 <sup>b</sup>	55 <sup>c</sup>	55 <sup>c</sup>	50 <sup>c</sup>	30 <sup>e</sup>	5 <sup>f</sup>	2 <sup>f</sup>	0 <sup>f</sup>	0 <sup>f</sup>	0 <sup>f</sup>	30 <sup>e</sup>	40 <sup>d</sup>	30 <sup>e</sup>	0 <sup>f</sup>	0 <sup>f</sup>	0 <sup>f</sup>	0 <sup>f</sup>
<i>P. camembertii</i> Thom ATCC 5860	80 <sup>a</sup>	75 <sup>a</sup>	72 <sup>b</sup>	70 <sup>b</sup>	60 <sup>c</sup>	40 <sup>d</sup>	41 <sup>d</sup>	30 <sup>e</sup>	30 <sup>e</sup>	20 <sup>f</sup>	20 <sup>f</sup>	3 <sup>g</sup>	0 <sup>g</sup>	35 <sup>e</sup>	35 <sup>e</sup>	30 <sup>e</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>
<i>P. chrysogenum</i> Thom ATCC 58611	85 <sup>a</sup>	84 <sup>a</sup>	80 <sup>a</sup>	75 <sup>b</sup>	45 <sup>c</sup>	40 <sup>c</sup>	45 <sup>c</sup>	35 <sup>f</sup>	25 <sup>f</sup>	20 <sup>g</sup>	25 <sup>f</sup>	30 <sup>f</sup>	0 <sup>h</sup>	30 <sup>f</sup>	45 <sup>c</sup>	30 <sup>f</sup>	0 <sup>h</sup>	0 <sup>h</sup>	0 <sup>h</sup>	0 <sup>h</sup>
<i>P. griseofulvum</i> Dierckx ATCC 11885	85 <sup>a</sup>	70 <sup>b</sup>	85 <sup>a</sup>	75 <sup>b</sup>	35 <sup>c</sup>	30 <sup>d</sup>	40 <sup>c</sup>	39 <sup>c</sup>	0 <sup>g</sup>	0 <sup>g</sup>	7 <sup>f</sup>	25 <sup>d</sup>	0 <sup>g</sup>	25 <sup>d</sup>	30 <sup>d</sup>	25 <sup>d</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>
<i>P. purpurogenum</i> Stoll ATCC 20204	87 <sup>a</sup>	87 <sup>a</sup>	80 <sup>b</sup>	65 <sup>c</sup>	40 <sup>d</sup>	20 <sup>f</sup>	60 <sup>c</sup>	18 <sup>g</sup>	6 <sup>h</sup>	8 <sup>h</sup>	20 <sup>f</sup>	0 <sup>j</sup>	0 <sup>j</sup>	22 <sup>f</sup>	30 <sup>c</sup>	20 <sup>f</sup>	0 <sup>j</sup>	0 <sup>j</sup>	0 <sup>j</sup>	0 <sup>j</sup>
<i>P. purpurogenum</i> Stoll ATCC 26940	92 <sup>a</sup>	87 <sup>a</sup>	82 <sup>b</sup>	77 <sup>b</sup>	25 <sup>e</sup>	25 <sup>e</sup>	30 <sup>d</sup>	60 <sup>c</sup>	5 <sup>f</sup>	7 <sup>f</sup>	10 <sup>f</sup>	35 <sup>d</sup>	0 <sup>g</sup>	20 <sup>c</sup>	30 <sup>d</sup>	25 <sup>e</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>	0 <sup>g</sup>

\*Values for each test fungus followed by the same letter in horizontal rows are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

Significant reduction in conidial germination of *Fusarium* species was observed in the presence of 100 µg/ml of PP at all pH levels. The lowest percentage in conidial germination was at pH 4 for the majority of the *Fusarium* species and pH 4 and 8 for *F. sporotrichioides*. At 200 µg/ml of PP, there was no conidial germination at any of the pH levels for the *Fusarium* species.

Conidial germination for *Penicillium* species was significantly reduced in the presence of 100 µg/ml of BHA at all pH levels. The lowest percent conidial germination was at pH 4 for *P. aurantiogriseum* ATCC 58603, *P. griseofulvum*, and *P. purpurogenum* ATCC 26940; pH 6 for *P. chrysogenum* and *P. purpurogenum* ATCC 20204; and pH 10 for *P. aurantiogriseum* ATCC 58604 and *P. camembertii*. Further reduction in conidial germination was

observed in presence of 200 µg/ml of BHA for species of *Penicillium*. The lowest percent germination observed was at pH 4 for *P. aurantiogriseum* ATCC 58603, pH 4 and 6 for *P. aurantiogriseum* ATCC 58604, *P. purpurogenum* ATCC 20204 and *P. purpurogenum* ATCC 26940, pH 6 for *P. chrysogenum*, pH 8 for *P. griseofulvum*, and pH 10 for *P. camembertii*. Complete inhibition of conidial germination was observed at pH 8 and 10 for *P. aurantiogriseum* ATCC 58604, pH 4 and 6 for *P. griseofulvum*, and pH 10 for *P. purpurogenum* ATCC 20204.

In the presence of 100 µg/ml of PP, the lowest percentage of conidial germination was recorded at pH 6 for the majority of the species of *Penicillium* with complete inhibition at pH 4. Conidial germination was completely inhibited, in the presence of 200 µg/ml of PP, for all of

TABLE 3. Percentages of reduction of colony diameter in *Fusarium* and *Penicillium* species caused by various concentrations of BHA and PP in potato dextrose agar at pH 4, 6, 8, and 10 after 7d of incubation at 27°C.

Fungi	% Reduction in colony diameter*															
	BHA/100				BHA/200				PP/100				PP/200			
	4	6	8	10	4	6	8	10	4	6	8	10	4	6	8	10
<i>F. graminearum</i> Schwabe ATCC 56749	19 <sup>b</sup>	42 <sup>f</sup>	100 <sup>a</sup>	40 <sup>f</sup>	66 <sup>d</sup>	85 <sup>b</sup>	50 <sup>e</sup>	100 <sup>a</sup>	75 <sup>c</sup>	53 <sup>e</sup>	38 <sup>e</sup>	63 <sup>d</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>F. moniliforme</i> Sheldon ATCC 38946	57 <sup>f</sup>	50 <sup>g</sup>	50 <sup>g</sup>	63 <sup>e</sup>	68 <sup>d</sup>	60 <sup>e</sup>	69 <sup>d</sup>	85 <sup>b</sup>	88 <sup>b</sup>	75 <sup>c</sup>	58 <sup>f</sup>	63 <sup>c</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>F. oxysporum</i> Schlechtendahl ATCC 46995	39 <sup>f</sup>	63 <sup>d</sup>	44 <sup>f</sup>	56 <sup>e</sup>	85 <sup>b</sup>	81 <sup>b</sup>	75 <sup>c</sup>	80 <sup>b</sup>	75 <sup>c</sup>	63 <sup>d</sup>	33 <sup>i</sup>	56 <sup>e</sup>	100 <sup>a</sup>	77 <sup>c</sup>	73 <sup>c</sup>	81 <sup>b</sup>
<i>F. poae</i> (Peck) Wollenweber ATCC 60317	69 <sup>c</sup>	44 <sup>f</sup>	39 <sup>g</sup>	50 <sup>e</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	83 <sup>b</sup>	38 <sup>e</sup>	0	63 <sup>d</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>F. roseum</i> Link ATCC 28114	79 <sup>c</sup>	62 <sup>d</sup>	50 <sup>f</sup>	91 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	63 <sup>d</sup>	56 <sup>e</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>F. sporotrichioides</i> Sherbakoff ATCC 24630	75 <sup>c</sup>	50 <sup>g</sup>	62 <sup>e</sup>	69 <sup>d</sup>	100 <sup>a</sup>	94 <sup>b</sup>	62 <sup>e</sup>	100 <sup>a</sup>	64 <sup>d</sup>	44 <sup>b</sup>	50 <sup>g</sup>	56 <sup>f</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>P. aurantiogriseum</i> Dierckx ATCC 58604	61 <sup>d</sup>	60 <sup>d</sup>	80 <sup>e</sup>	42 <sup>e</sup>	90 <sup>b</sup>	87 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>	6 <sup>g</sup>	8 <sup>g</sup>	25 <sup>f</sup>	25 <sup>f</sup>	81 <sup>c</sup>	23 <sup>f</sup>	42 <sup>e</sup>	40 <sup>e</sup>
<i>P. aurantiogriseum</i> Dierckex ATCC 58603	40 <sup>g</sup>	44 <sup>g</sup>	45 <sup>f</sup>	100 <sup>a</sup>	90 <sup>b</sup>	85 <sup>c</sup>	92 <sup>b</sup>	100 <sup>a</sup>	100	67 <sup>d</sup>	50 <sup>f</sup>	60 <sup>e</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>P. camembertii</i> Thom ATCC 58608	8 <sup>g</sup>	40 <sup>e</sup>	40 <sup>e</sup>	25 <sup>f</sup>	58 <sup>c</sup>	50 <sup>d</sup>	67 <sup>b</sup>	40 <sup>e</sup>	100 <sup>a</sup>	58 <sup>c</sup>	30 <sup>f</sup>	60 <sup>e</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>P. chrysogenum</i> Thom ATCC 58611	57 <sup>d</sup>	67 <sup>c</sup>	45 <sup>e</sup>	40 <sup>f</sup>	71 <sup>b</sup>	71 <sup>b</sup>	75 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>	67 <sup>c</sup>	46 <sup>e</sup>	67 <sup>c</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>P. griseofulvum</i> Dierckx ATCC 11885	70 <sup>c</sup>	67 <sup>d</sup>	50 <sup>e</sup>	43 <sup>f</sup>	100 <sup>a</sup>	100 <sup>a</sup>	87 <sup>b</sup>	44 <sup>f</sup>	100 <sup>a</sup>	73 <sup>c</sup>	50 <sup>e</sup>	70 <sup>c</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>P. purpurogenum</i> Stoll ATCC 20204	61 <sup>c</sup>	80 <sup>c</sup>	33 <sup>f</sup>	86 <sup>b</sup>	89 <sup>b</sup>	88 <sup>b</sup>	73 <sup>d</sup>	100 <sup>a</sup>	100 <sup>a</sup>	84 <sup>b</sup>	71 <sup>d</sup>	78 <sup>c</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>
<i>P. purpurogenum</i> Stoll ATCC 26940	75 <sup>c</sup>	73 <sup>d</sup>	60 <sup>f</sup>	0 <sup>g</sup>	92 <sup>b</sup>	91 <sup>b</sup>	87 <sup>b</sup>	79 <sup>c</sup>	100 <sup>a</sup>	71 <sup>d</sup>	67 <sup>c</sup>	73 <sup>d</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>

\*Percent reduction = 100 - (diameter on medium with phenolic antioxidant/diameter on PDA).

\*Values for each test fungus followed by the same letter in horizontal rows are not significantly different (P = 0.05) according to Duncan's multiple range test.

*Penicillium* species with the exception of *P. aurantiogriseum* ATCC 24630 where conidial germination was only completely inhibited at pH 10.

The effects of BHA and PP on colony diameter of *Fusarium* and *Penicillium* species at four pH levels are shown in Table 3. In the presence of 100 µg/ml of BHA, the highest reduction in colony diameter was at pH 4 for *F. poae* and *F. sporotrichioides*, pH 6 for *F. graminearum* and *F. oxysporum*, and pH 10 for *F. moniliforme* and *F. roseum*. Only *F. graminearum* was completely inhibited at pH 8. At each pH level in the presence of 200 µg/ml, there was further reduction in the colony diameter of *Fusarium* species. The highest insignificant reduction in colony diameter was at pH 4, 6, and 10 for *F. oxysporum*; pH 6 for *F. graminearum* and *F. sporotrichioides*; and pH 10 for *F.*

*moniliforme*. At pH 4, 6, 8, and 10, only *F. poae* and *F. roseum* were completely inhibited, while *F. graminearum* was inhibited at pH 10 and *F. sporotrichioides* at pH 4 and 10.

Percent reduction in colony diameter was highest at 100 µg/ml of PP at pH 4 for the majority of the *Fusarium* species. *F. sporotrichioides* was completely inhibited at pH 4 and 10. The majority of the *Fusarium* species were completely inhibited at the four pH levels when PP was increased to 200 µg/ml, with the exception of *F. oxysporum*. *F. oxysporum* was completely inhibited only at pH 4.

In the presence of 100 µg/ml of BHA, the highest reduction in colony diameter was at pH 4 for *P. griseofulvum* and *P. purpurogenum* ATCC 26940, pH 6 and 8 for *P. camembertii*, pH 6 for *P. aurantiogriseum* ATCC 58603

and *P. aurantiogriseum*, and pH 10 for *P. purpurogenum* ATCC 20204. Only *P. aurantiogriseum* ATCC 58604 was completely inhibited at pH 10. When BHA was increased to 200 µg/ml, the highest reduction in colony diameter was observed at pH 4 and 6 for *P. aurantiogriseum* ATCC 58603 and *P. purpurogenum* ATCC 20204; pH 4, 6, and 8 for *P. chrysogenum* and *P. purpurogenum* ATCC 26940; and pH 8 for *P. camembertii* and *P. griseofulvum*.

In the presence of 100 µg/ml of PP, the highest reduction in colony diameter occurred at pH 8 and 10 for *P. aurantiogriseum* ATCC 68603; pH 6 for *P. aurantiogriseum* ATCC 58604, *P. griseofulvum*, and *P. purpurogenum* ATCC 20204; pH 6 and 10 for *P. chrysogenum* and *P. purpurogenum* ATCC 26940; and pH 10 for *P. camembertii*. At pH 4, all *Penicillium* species were completely inhibited. When PP was increased to 200 µg/ml, all *Penicillium* species were completely inhibited at the four pH levels.

### DISCUSSION

It is generally accepted that most fungi grow over the pH range 3 to 8 (14). The toxigenic fungi used in this investigation grew at all four pH levels at 27°C, which falls in the range reported for toxigenic fungi by Wheeler et al. (14). The pH range reported for growth rates of the two genera of toxigenic fungi, in their study was from 2 to 11.

*Fusarium* species were tolerant of both acid and basic pH, except for *F. graminearum* which showed a slightly lower capability for growth at pH 4 and was more affected by basic pH levels. The optimum pH for growth of *F. graminearum* was in agreement with the pH reported by Booth (3).

*Penicillium* species grew over the four pH levels. However, reduction in growth of the majority of the species was below pH 5. This is in agreement with the findings of Brancoto and Golding (5), Holmquist et al. (9), and Sacks et al. (12).

PP was more effective than BHA in inhibiting conidia germination and mycelial growth of the test fungi. However, PP exhibited a greater inhibitory effect against *Fusarium* species.

The fungitoxic effect of BHA on conidial germination was most pronounced at the lower and higher pH levels for *Fusarium* species at 100 µg/ml of BHA. Similar results were observed for some species of *Fusarium* at 200 µg/ml. On the other hand, four species were completely inhibited at all of the pH levels.

The fungitoxic effect of BHA and PP on conidial germination of *Penicillium* species varied. However, for the majority of the species, the most pronounced effect at 200 µg/ml of BHA was recorded at pH 4 and 6. In the presence of 100 µg/ml of PP, the most pronounced effect on conidial germination was at pH 4 and at all pH levels for all of the species at 200 µg/ml with the exception of one.

In the presence of BHA and PP (100 µg/ml), there was reduction in colony diameter of *Fusarium* species. While

greater reductions were observed in the presence of 200 µg/ml of BHA, there were more *Fusarium* species completely inhibited at all pH levels in the presence of 200 µg/ml of PP. A similar pattern in reduction of colony diameters and complete inhibition of mycelial growth was observed for the majority of the *Penicillium* species.

The results obtained in this study support the fact that pH is an important physical factor that must be addressed in fungitoxic studies of antioxidants. This is important since mycelial growth and conidial germination, two distinct phases in the asexual cycle of toxigenic fungi, are affected.

Information on the fungitoxic activity of phenolic antioxidants in combination with physical and chemical factors helps to determine the overall potential effectiveness against toxigenic fungi. Additional research on the effectiveness of phenolic antioxidants in combination with physical and chemical factors would be useful in defining the maximal antifungal activity of selected phenolic antioxidants.

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