# Synergistic Effect of Ethazole and Pentachloronitrobenzene on Inhibition of Growth and Reproduction of *Pythium aphanidermatum*

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**ABSTRACT**

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Quintozene (a mixture of ethazole and pentachloronitrobenzene [PCNB]) prevented mycelial growth, oospore germination, sporangium formation, and zoospore production in *Pythium aphanidermatum*. Ethazole alone was less effective, and PCNB had no effect. A 1:5 mixture of ethazole and PCNB at ≥50 ppm (w/w, a.i.) inhibited mycelial growth similar to the inhibition produced by quintozene alone.

Additional key words: cucurbit root rot and damping-off, fungicide

Since 1960, several fungicides have been used to control *Pythium aphanidermatum* (Edson) Fitz. in various crops (1,4,7,8). Seed treated with captan or thiram provides good emergence and stands of peas in fields in Iran (3). Soil drenches of ethazole are generally more effective and safer than fenaminosulf drenches for controlling root rot of chrysanthemum incited by *P. aphanidermatum*, *P. debaryanum*, and *P. ultimum* (1). Treatment with an aqueous suspension of ethazole has resulted in a good stand of tomato seedlings with a low incidence of disease caused by *P. aphanidermatum* (7). Seed treatment with quintozene effectively prevents seed root, seedling damping-off, and death of mature cantaloupe plants incited by *P. aphanidermatum* during 10 wk in the greenhouse (5). Ethazole is less effective than quintozene, and pentachloronitrobenzene (PCNB) has no effect on disease control (5).

## MATERIALS AND METHODS

The effect of quintozene (a mixture of ethazole and PCNB), ethazole, and PCNB were tested on vegetative and reproductive stages of *P. aphanidermatum*.

For mycelial growth and germination studies, the commercial formulation of each fungicide was mixed with corn meal agar (CMA) before pouring plates. The final concentrations of each fungicide were 5, 10, 50, and 100 ppm (a.i.). Ethazole and PCNB were also combined at 1:5, 1:10, and 1:20 ratios (w/w, a.i.) in CMA at 5, 10, 50, and 100 ppm for each mixture. CMA without fungicide was used as control (0 ppm). A 5-mm block of a two-day-old culture of the pathogen on CMA was placed in the center of each 9-cm petri plate and incubated at 35 C in the dark for as long as 7 days; radial growth was measured daily.

Two-month-old oospores, grown in the dark on cleared V-8 broth at 25 C (2), were used. The culture was frozen at −20 C for 24 hr to destroy mycelial fragments. The mycelial mats containing oospores were thawed, ground in a sterilized mortar with a few milliliters of sterile distilled water, and passed through several layers of cheesecloth. Free oospores were collected in the filtrate and spread on fungicide-amended CMA. After 1-day incubation in the dark at 35 C, 100 oospores from each plate were examined.

Sporangia of *P. aphanidermatum* were obtained as reported earlier (6). The fungus was cultured on V-8 agar, incubated at 35 C under 1,200 lux of cool white fluorescent illumination for 2 days, and cut into 5-mm strips. Half of the strips in each plate were placed in a sterile petri dish and sterile distilled water was added. The water was removed 30 min later and replaced by 20 ml of the 0, 5, 10, 50, or 100 ppm fungicide suspension. Plates were then incubated at 35 C under 1,200 lux of cool white fluorescent illumination. Sporangia were counted under the microscope 24 hr later.

The remaining portion of the culture was used to determine zoospore production. After sporangial formation, the water was removed and replaced with 20

**Table 1.** Effect of fungicide formulations on mycelial growth of *Pythium aphanidermatum* on cornmeal agar at 35 C

| Fungicide | Rate (ppm, a.i.) | Radial growth (mm) after incubation | | |
|---|---|---|---|---|
| | | 2 days | 5 days | 7 days |
| Quintozene | 100 | 0 | 0 | 0 |
| | 50 | 0 | 0 | 0 |
| | 10 | 0 | 53 | 85 |
| | 5 | 14 | 85 | ...[a] |
| Ethazole | 100 | 0 | 18 | 46 |
| | 50 | 0 | 34 | 85 |
| | 10 | 45 | 85 | ... |
| | 5 | 85 | ... | ... |
| PCNB[b] | 100 | 85 | ... | ... |
| | 50 | 85 | ... | ... |
| | 10 | 85 | ... | ... |
| | 5 | 85 | ... | ... |
| Ethazole/PCNB, 1:5[c] | 100 | 0 | 0 | 0 |
| | 50 | 0 | 0 | 0 |
| | 10 | 30 | 34 | 41 |
| | 5 | 85 | ... | ... |
| Ethazole/PCNB, 1:10 | 100 | 0 | 5 | 5 |
| | 50 | 4 | 30 | 33 |
| | 10 | 85 | ... | ... |
| | 5 | 85 | ... | ... |
| Ethazole/PCNB, 1:20 | 100 | 10 | 15 | 17 |
| | 50 | 40 | 75 | 80 |
| | 10 | 85 | ... | ... |
| | 5 | 85 | ... | ... |
| Control | 0 | 85 | ... | ... |

[a] Not measurable (>85 mm).
[b] PCNB = pentachloronitrobenzene.
[c] Ratio is based on active ingredient. The final rate (ppm) is the sum of the active ingredients of both fungicides.

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**Table 2.** Effect of quintozene, ethazole, and pentachloronitrobenzene (PCNB) on oospore germination,[a] sporangium, and zoospore production in *Pythium aphanidermatum*

| Fungicide | Rate[b] (ppm) | Germinated oospore[c] (%) | Sporangia[c] (no./×10 field) | Zoospores/ml[c] (×10⁶) |
|---|---|---|---|---|
| Quintozene | 100 | 0.0 | 0.0 | 0.0 |
| | 50 | 0.0 | 0.0 | 0.0 |
| | 10 | 4.6 | 1.3 | 0.3 |
| | 5 | 20.6 | 8.4 | 1.6 |
| Ethazole | 100 | 0.0 | 0.0 | 1.0 |
| | 50 | 2.0 | 1.3 | 1.0 |
| | 10 | 34.3 | Many[d] | 1.2 |
| | 5 | 54.3 | Many | 1.9 |
| PCNB | 100 | 53.3 | Many | 0.6 |
| | 50 | 54.6 | Many | 1.0 |
| | 10 | 49.0 | Many | 2.0 |
| | 5 | 52.6 | Many | 2.1 |
| Control | 0 | 54.0 | Many | 2.1 |

[a] Two-month-old oospores grown in V-8 broth at 25 C in the dark.
[b] Rate is based on active ingredient.
[c] Average of three replicates.
[d] Too many to count.

ml of the 0, 5, 10, or 100 ppm fungicide suspension. To induce zoospore production, plates were incubated in the dark at 20 C for 4 hr (6). Zoospores were immobilized on a vortex mixer and counted by using a hemocytometer (eight counts per plate).

For the zoospore germination study, a suspension of motile zoospore was spread on 1.6% water agar containing the 0, 5, 10, 50, 100, or 500 ppm fungicide suspension and incubated in the dark at 35 C; 100 zoospores were examined in each replicate plate 24 hr later. Three replicates of each treatment were used in all experiments.

## RESULTS AND DISCUSSION

Quintozene, ≥50 ppm, inhibited growth of the pathogen. Ethazole, ≥100 ppm, did not prevent growth of the pathogen, and PCNB at any concentration did not inhibit mycelial growth (Table 1). Mycelial growth of *P. aphanidermatum* was inhibited similarly by quintozene alone and by the 1:5 ratio of ethazole/PCNB at 50 ppm or higher (Table 1). As the concentration of PCNB in the mixture increased, the inhibitory effect decreased. PCNB did not control damping-off in cantaloupe (5) and did not affect mycelial growth of the pathogen, but it had a synergistic effect when mixed with ethazole. The mechanism responsible for the synergistic effect was not investigated.

Quintozene reduced oospore germination at all concentrations tested and inhibited oospore germination at 50 ppm or higher. Ethazole was less effective than quintozene, especially at lower concentrations. PCNB at any concentration did not inhibit oospore germination (Table 2).

No sporangia were formed at a 50 ppm or higher concentration of quintozene. Ethazole at 100 ppm inhibited sporangial production, and none of the concentration of PCNB inhibited sporangial production (Table 2). No zoospores were produced at a concentration ≤50 ppm of quintozene. Ethazole and PCNB did not significantly affect zoospore production at any concentration (Table 2). Quintozene effectively prevented damping-off incited by *P. aphanidermatum* in cantaloupe, but ethazole was less effective (5). Zoospore germination was not greatly reduced by any fungicide in concentrations to 100 ppm. At 500 ppm, however, germination was 0, 26, and 98% with quintozene, ethazole, and PCNB, respectively.

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