

In Vitro Suppression of Soilborne Plant Pathogens by Coir

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SUMMARY. Experiments conducted in vitro showed that coir [coconut (*Cocos nucifera*) mesocarp pith] suppressed growth of soilborne plant pathogens. Mycelial growth of *Phytophthora capsici* on potato dextrose agar (PDA) amended with an unsterilized coir suspension was strongly inhibited regardless of suspension concentration. Growth of *P. capsici* on PDA amended with a filter-sterilized coir suspension was uninhibited. Growth of *Fusarium solani* on water agar (WA) amended with unautoclaved coir was completely inhibited. Growth of *F. solani* on WA amended with autoclaved coir was uninhibited. *Aspergillus terreus* recovered from coir inhibited mycelial growth of various soilborne pathogens by up to 75%. The results from this study suggest that coir has the ability to suppress soilborne plant pathogens in vitro and this ability is largely due to microorganisms associated with the substrate.

Coir has become popular as a potting medium in greenhouse and nursery production. Qualities of coir that make it a good peat substitute include high water holding capacity, excellent drainage, physical resilience, and the fact that it is a renewable resource (Masago et al., 1977; Meerow, 1994; Prasad, 1997). It has been demonstrated that coir can suppress certain soilborne plant pathogens (Candole and Evans, 2004; van der Gaag and Wever, 2005). Combining the disease-suppressive properties of coir with other management strategies such as fungicide application in an integrated pest management program may result in more effective and less costly management of soilborne diseases in greenhouse and nursery production.

Suppressive substrates reduce soilborne diseases by their physical and/or chemical properties such as drainage, pH, and chemical inhibitors (Hoitink and Fahy, 1986; van der Gaag and Wever, 2005) or by supporting beneficial microorganisms

that lower disease incidence or severity by mechanisms such as competition, antibiosis, parasitism, or induction of systemic resistance (Han et al., 1998; Hoitink and Boehm, 1999). In addition to coir, soil substitutes such as peat and hardwood bark also have been shown to suppress certain plant diseases (Hoitink and Fahy, 1986). Unautoclaved rockwool recolonized by indigenous microflora was suppressive to *Pythium aphanidermatum* (Postma et al., 2000).

Horticultural studies suggest that coir may be a suitable organic component in soilless media and is better than most other alternatives, including rock wool (Islam et al., 2002; Meerow, 1994; Meerow, 1995). Trials using tomato (*Solanum lycopersicum*) as a test crop indicated that coir gave better crop productivity than rockwool (Islam et al., 2002). Population densities of *Phytophthora capsici*, *P. nicotianae*, *P. aphanidermatum*, and *P. ultimum* were reduced by 76%, 80%, 32%, and 11%, respectively, when tomatoes were grown in coir compared with peat.

In the same study, damping-off incidence was reduced by 88%, 90%, and 41% for *P. capsici*, *P. nicotianae*, and *P. aphanidermatum*, respectively (Candole and Evans, 2004). Research has not been done to determine what causes coir to suppress plant pathogens. The objective of this study was to evaluate, in vitro, the ability of coir to suppress soilborne plant pathogens.

Materials and methods

FUNGAL CULTURES. *Fusarium solani* was isolated from infected gerbera (*Gerbera jamesonii*) seedlings and maintained on modified Nash-Snyder medium (Nash and Snyder, 1962). *P. capsici* was recovered from recycled nursery water and maintained on a *Phytophthora*-selective (PARPH) medium (Masago et al., 1977; Tsao and Guy, 1977). Cultures of *P. parasitica*, *P. capsici*, *P. citricola*, *P. cinnamomi*, *P. cactorum*, and *P. cryptogea* were provided by M. Stanghellini (University of California, Riverside). *Sclerotinia sclerotiorum*, *Rhizoctonia* sp., *Cylindrocladium* sp., *Pythium* sp., and *Verticillium* sp. were isolated from a variety of infected greenhouse, nursery, and landscape plants brought to S. Wegulo's laboratory (University of California, Riverside) for diagnosis. All cultures except *F. solani* and *Phytophthora* species were maintained on potato dextrose agar (PDA) in 9-cm-diameter petri plates and were stored at 4 °C until needed for experiments. Fungal cultures used in all experiments were grown on PDA, half-strength PDA (1/2-PDA), or water agar (WA) in 9-cm-diameter petri plates.

SOURCE OF COIR. The coir used in this study was obtained from a commercial supplier (Cal-Native Plants, Menifee Valley, CA). Coconut husk was collected from areas surrounding the city of Tecoman, state of Colima, Mexico, processed into

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI units	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
3.7854	gal	L	0.2642
2.54	inch(es)	Cm	0.3937
25.4	inch(es)	Mm	0.0394
1	micron	µm	1
28.3495	oz	g	0.0353
(°F - 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

coir in the same city, and then shipped to F. Chavez (Cal-Native Plants). Coir used in all experiments was not retted. Retting is a process used to extract quality coir fiber from coconut husk (Neena et al., 2007). It involves soaking coconut husk in water to decompose the binding materials that hold the fibers together in the husk (Zachariah and Muralidharan, 2002).

EVALUATION OF MYCELIAL GROWTH OF *P. CAPSICI* ON MEDIUM AMENDED WITH A COIR SUSPENSION. Seventy-two grams of dry coir was soaked in 1 L of distilled water and set on a laboratory bench for 3, 6, 9, or 12 d at room temperature. For each soaking period, 300 mL of suspension was collected by filtering the suspension through several layers of cheesecloth. One half of the suspension was filter-sterilized with a 0.2- μ m filter membrane and the other half was left unsterilized. A 7-mm-diameter plug of *P. capsici* from the edge of an actively growing culture was transferred to the center of each petri plate (experimental unit) with 15 mL of PDA amended with the suspension at 0.1, 0.2, and 0.4 mL of suspension per milliliter of PDA. For each concentration, the suspension was measured with a micropipette and transferred to autoclaved PDA that had cooled down to ≈ 40 °C in a laminar flow hood. A magnetic stirrer was used to mix the suspension with the PDA. Petri plates were incubated at 22 °C in a 12-h light-dark cycle. The experimental design was a split-split plot in randomized complete blocks with three replications. Suspension concentrations were the main plots, filter-sterilization treatments (sterilized and unsterilized) were the subplots, and soaking periods were the sub-subplots. Mycelial radius was measured 6 d after initiation of cultures and was used to calculate mycelium-covered area. The experiment was conducted twice.

EVALUATION OF MYCELIAL GROWTH OF *F. SOLANI* ON COIR-AMENDED MEDIUM. Dry coir was ground to powder using a No. 1 Wiley mill (Arthur H. Thomas, Philadelphia, PA) with a 1-mm-diameter screen. Five grams of autoclaved or unautoclaved ground coir, 0.25 g of potassium chloride, 0.005 g of ferrous sulfate, 5 g of yeast extract, 0.25

g of magnesium sulfate, 0.25 g of calcium nitrate, and 0.125 g of mono-potassium phosphate were incorporated with a magnetic stirrer into 500 mL of WA that had cooled down to ≈ 40 °C in a laminar flow hood. Twenty milliliters of the amended WA was poured into each of four petri plates. WA amended with 5 g of peat or 5 g of malt extract was similarly prepared. There were six treatments in the experiment: WA amended with autoclaved coir, WA amended with unautoclaved coir, WA amended with autoclaved peat, WA amended with unautoclaved peat, WA amended with autoclaved malt extract, and WA amended with unautoclaved malt extract. The malt extract treatments served as controls. Peat was included because microbial antagonists have been shown to be associated with it (Hoitink and Boehm, 1999; Hoitink and Fahy, 1986). A 7-mm-diameter plug of *F. solani* from the edge of an actively growing culture was placed in the center of each petri plate with the amended medium. The plates were incubated at 25 °C in a 12-h light-dark cycle. Mycelial radius was measured 7 d after initiation of cultures and was used to calculate mycelium-covered area. The experimental design was a split plot in randomized complete blocks with four replications. Autoclave treatments served as the main plots and substrates (coir, peat, and malt extract) served as the subplots. The experiment was conducted three times.

INHIBITION OF MYCELIAL GROWTH OF SOILBORNE PATHOGENS BY *ASPERGILLUS TERREUS*. In the course of this study, *A. terreus* was consistently isolated from coir. As a contaminant, it was observed to inhibit mycelial growth of *P. capsici* and *F. solani*. An experiment was conducted to investigate the inhibitory effect of *A. terreus* on 12 soilborne plant pathogens. The pathogens were *P. parasitica*, *P. capsici*, *P. citricola*, *P. cinnamomi*, *P. cactorum*, *P. cryptogea*, *F. solani*, *S. sclerotiorum*, *Rhizoctonia* sp., *Cylindrocladium* sp., *Pythium* sp., and *Verticillium* sp.

An isolate of *A. terreus* was recovered from coir by sprinkling ground coir dust on 1/2-PDA. Cultures of the fungus were transferred to separate petri plates, each containing 15 mL of 1/2-PDA. A 7-mm-

diameter plug of this isolate was placed near the edge of a petri plate containing 15 mL of 1/2-PDA. A 7-mm-diameter plug of each of the 12 test pathogens was taken from the edge of an actively growing culture and placed near the opposite edge of the petri plate containing an *A. terreus* plug. Petri plates were maintained in an incubator at 22 °C with a 12-h light-dark cycle. The distance from the plug of each pathogen to the edge of the zone of inhibition (caused by *A. terreus*) or the edge of the *A. terreus* culture was measured 14 d after initiation of cultures.

Each pathogen also was concurrently grown on control 1/2-PDA plates without an *A. terreus* plug. A 7-mm-diameter plug of each pathogen was placed on the edge of a petri plate and the distance from the plug to the actively growing edge of the mycelial culture was measured 14 d after initiation of cultures. The percentage of inhibition of growth of each pathogen was calculated as $[(a - b)/a] \times 100$, where a is the distance from the 7-mm-diameter plug of the pathogen at the edge of the control plate to edge of the mycelial culture and b is the distance from the 7-mm-diameter plug of the pathogen at the edge of the plate with an *A. terreus* plug to the edge of the zone of inhibition (caused by *A. terreus*) or the edge of the *A. terreus* culture. The test pathogens served as treatments and were arranged in randomized complete blocks with three replications (three plates with *A. terreus* plus the pathogen and three control plates with the pathogen alone). The experiment was conducted twice.

DATA ANALYSIS. The GLM procedure of SAS (version 8; SAS Institute, Cary, NC) was used to analyze data. Data from replicate experiments were combined or not combined based on homogeneity or heterogeneity, respectively, of error variances determined from the F -ratio test or Bartlett's test (Gomez and Gomez, 1984). The mycelium-covered area was calculated as $\pi \times R^2 - \pi \times r^2$, where R is the radius of the fungal culture in the 9-cm-diameter petri plate and r is the radius of the 7-mm-diameter mycelial plug placed at the center of the petri plate. Fisher's least significant difference test at $P = 0.05$ (Gomez and Gomez, 1984) was

used to compare pairs of treatment means.

Results

EVALUATION OF MYCELIAL GROWTH OF *P. CAPSICI* ON MEDIUM AMENDED WITH A COIR SUSPENSION. In Expt. 1, *F* values for coir suspension concentration ($P < 0.0001$), filtration ($P < 0.0001$), soaking period ($P < 0.0084$), and concentration by filtration interaction ($P < 0.001$) were highly significant, whereas *F* values for concentration by soaking period, filtration by soaking period, and the three-way interaction were not significant ($P > 0.05$). In Expt. 2, *F* values for all three treatments and their interactions were highly significant ($P < 0.0009$), except for the three-way interaction ($P = 0.0832$).

The unsterilized coir suspension strongly inhibited mycelial growth of *P. capsici* at all concentrations in Expts. 1 and 2 (Table 1). In the filter-sterilized coir suspension treatments in Expts. 1 and 2, there was no significant difference in mycelium-covered area between the lowest (0.1 mL suspension per milliliter of PDA) and second lowest (0.2 mL suspension per milliliter of PDA) concentrations of the suspension. However, the mycelium-covered area was significantly ($P = 0.05$) greater at the highest concentration (0.4 mL suspension per milliliter of PDA) compared with the two lower concentrations (Table 1).

The effect of soaking period on mycelial growth of *P. capsici* varied among coir suspension concentrations (Table 2). At the lowest concentration (0.1 mL suspension per milliliter of PDA), there were no significant differences in the mycelium-covered area among soaking periods in Expts. 1 and 2. At the second lowest concentration (0.2 mL suspension per milliliter of PDA), the mycelium-covered area in the 6-d soaking period was significantly ($P = 0.05$) greater than the mycelium-covered area in the 3-d soaking period in both experiments. At the highest concentration (0.4 mL suspension per milliliter of PDA), the mycelium-covered area in the 9-d soaking period was significantly ($P = 0.05$) less than the mycelium-covered area in the 6-d soaking period in both experiments.

In the unsterilized coir suspension treatment, mycelial growth was strongly inhibited in Expts. 1 and 2, regardless of soaking period (Table 3). There were no significant differences at $P = 0.05$ in the mycelium-covered area among soaking periods in both experiments; however, the mycelium-covered area was less in the 9- and 12-d compared with the 3- and 6-d soaking periods. Colonies of various microorganisms were visible by day 7 after initiation of cultures. Three of these microorganisms were identified as *A. terreus*, *Paecilomyces* sp., and *Trichoderma* sp. The rest of the microorganisms were not identified.

In the filter-sterilized coir suspension treatment, mycelial growth was

uninhibited (Table 3). Six days after initiation of cultures, the mycelium-covered area was significantly greater ($P = 0.05$) in the 6-d soaking period than in all other soaking periods in both experiments (Table 3).

EVALUATION OF MYCELIAL GROWTH OF *F. SOLANI* ON COIR-AMENDED MEDIUM. *F* values for the autoclave (autoclaved and unautoclaved) and media (coir, peat, and malt extract) treatments and their interaction were highly significant ($P < 0.0001$) in all three experiments. Unautoclaved coir and peat media completely inhibited the growth of *F. solani* (Table 4). Colonies of various microorganisms, including bacteria and fungi, were visible on plates

Table 1. Mycelium-covered area of *Phytophthora capsici* on potato dextrose agar (PDA) amended with an unsterilized or a filter-sterilized coir suspension at three concentrations.

Suspension concn (mL·mL ⁻¹ PDA)	Mycelium-covered area (mm ²) ^z					
	Sterilization: Expt. 1			Sterilization: Expt. 2		
	Unsterilized	Filter-sterilized	LSD ^y	Unsterilized	Filter-sterilized	LSD
0.1	1.3	30.0	1.8	1.3	31.6	2.3
0.2	1.2	31.4	1.8	1.3	30.7	2.3
0.4	1.9	41.7	1.8	2.1	43.3	2.3
LSD	1.7	1.7	—	1.8	1.8	—

^zMycelium-covered area was measured 6 d after initiation of cultures; 1 mm² = 0.0016 inch².

^yFisher's least significant difference at $P = 0.05$.

Table 2. Mycelium-covered area of *Phytophthora capsici* on potato dextrose agar (PDA) amended with a coir suspension at three concentrations and four soaking periods in distilled water.

Suspension concn (mL·mL ⁻¹ PDA)	Mycelium-covered area (mm ²) ^z									
	Soaking period (d): Expt. 1					Soaking period (d): Expt. 2				
	3	6	9	12	LSD ^y	3	6	9	12	LSD
0.1	16.1	16.0	14.8	15.5	2.1	16.6	17.0	15.8	16.3	1.3
0.2	15.4	18.0	16.0	15.8	2.1	14.8	17.3	15.4	16.5	1.3
0.4	22.5	23.4	20.4	20.9	2.1	23.2	24.5	21.3	21.8	1.3
LSD	2.2	2.2	2.2	2.2	—	1.4	1.4	1.4	1.4	—

^zMycelium-covered area was measured 6 d after initiation of cultures; 1 mm² = 0.0016 inch².

^yFisher's least significant difference at $P = 0.05$.

Table 3. Mycelium-covered area of *Phytophthora capsici* on potato dextrose agar amended with an unsterilized or a filter-sterilized coir suspension that formed during four soaking periods in distilled water.

Sterilization	Mycelium-covered area (mm ²) ^z									
	Soaking period (d): Expt. 1					Soaking period (d): Expt. 2				
	3	6	9	12	LSD ^y	3	6	9	12	LSD
Unsterilized	1.8	1.8	1.1	1.1	1.7	2.0	2.0	1.1	1.1	1.0
Filter-sterilized	34.2	36.6	33.1	33.6	1.7	34.4	37.3	34.0	35.3	1.0
LSD	1.8	1.8	1.8	1.8	—	1.6	1.6	1.6	1.6	—

^zMycelium-covered area was measured 6 d after initiation of cultures; 1 mm² = 0.0016 inch².

^yFisher's least significant difference at $P = 0.05$.

Table 4. Mycelium-covered area of *Fusarium solani* on water agar amended with autoclaved or unautoclaved coir, peat, or malt.

Autoclave treatment	Mycelium-covered area (mm ²) ^z											
	Expt. 1				Expt. 2				Expt. 3			
	Coir	Peat ^y	Malt ^x	LSD ^w	Coir	Peat	Malt	LSD ^w	Coir	Peat	Malt	LSD ^w
Autoclaved	38.1	37.5	35.7	1.1	38.1	38.1	37.8	0.2	36.4	38.1	38.1	3.7
Unautoclaved	0.0	0.0	37.0	1.1	0.0	0.0	38.1	0.2	0.0	0.0	37.0	3.7
LSD	1.4	1.4	1.4	—	0.3	0.3	0.3	—	3.8	3.8	3.8	—

^zMycelium-covered area was measured 7 d after initiation of cultures; 1 mm² = 0.0016 inch².

^yPeat was included because microbial antagonists have been shown to be associated with it.

^xMalt served as a control.

^wFisher's least significant difference at *P* = 0.05.

amended with unautoclaved coir and peat within 7 d of setting up the experiments. Autoclaved coir and peat media and autoclaved and unautoclaved malt media did not inhibit growth of *F. solani* (Table 4).

INHIBITION OF MYCELIAL GROWTH OF SOILBORNE PATHOGENS BY *A. TERREUS*. A clear zone of inhibition was observed between mycelia of *A. terreus* and mycelia of *S. sclerotiorum*, *Rhizoctonia* sp., *Cylindrocladium* sp., *Pythium* sp., and *Verticillium* sp. This zone was not apparent in the *A. terreus*/*Phytophthora* spp. cultures. *A. terreus* inhibited mycelial growth of the pathogens by up to 75% (Table 5). The least inhibition (18%) occurred on *Verticillium* sp.,

Table 5. Inhibition of soilborne plant pathogens by *Aspergillus terreus* recovered from coir.

Pathogen	Inhibition (%) ^z
<i>Phytophthora parasitica</i>	75
<i>Phytophthora capsici</i>	70
<i>Phytophthora citricola</i>	67
<i>Phytophthora cinnamomi</i>	65
<i>Phytophthora cactorum</i>	56
<i>Fusarium solani</i>	55
<i>Phytophthora cryptogea</i>	46
<i>Sclerotinia sclerotiorum</i>	42
<i>Rhizoctonia</i> sp.	37
<i>Cylindrocladium</i> sp.	37
<i>Pythium</i> sp.	23
<i>Verticillium</i> sp.	18
LSD ^y	15

^zMean of two experiments. The percentage of inhibition was based on linear measurement of pathogen cultures on half-strength potato dextrose agar plates with *A. terreus* compared with linear measurement of pathogen cultures on plates without *A. terreus*.

^yFisher's least significant difference at *P* = 0.05.

whereas *Phytophthora* species were inhibited the most (Table 5).

Discussion

PDA amended with an unsterilized coir suspension strongly suppressed growth of *P. capsici*, regardless of suspension concentration, but supported growth of several microorganisms. Suppression of *P. capsici* was likely due to competition by these microorganisms for space and nutrients. Although chemical inhibition of *P. capsici* was not investigated, it apparently played little or no role in suppression because PDA amended with a filter-sterilized coir suspension did not inhibit growth of the pathogen at all concentrations of the suspension. The facts that microorganisms did not grow on PDA amended with a filter-sterilized coir suspension and growth of *P. capsici* on this medium was not inhibited imply that microorganisms played a significant role in pathogen suppression on PDA amended with an unsterilized coir suspension.

It was interesting to note that amending PDA with the highest concentration of the filter-sterilized coir suspension (0.4 mL suspension per milliliter of PDA) resulted in significantly greater (*P* = 0.05) *P. capsici* mycelium-covered area than amending PDA with lower concentrations of the suspension (0.1 or 0.2 mL suspension per milliliter of PDA; Table 1). It is likely that the coir suspension contained nutrients used by *P. capsici*. The greater mycelium-covered area at the highest coir suspension concentration compared with the two lower concentrations was likely due to a higher concentration of nutrients per unit volume of PDA.

Regardless of soaking period, the lowest concentration (0.1 mL

suspension per milliliter of PDA) of unsterilized coir suspension effectively inhibited the growth of *P. capsici*. This implies that the population of microorganisms was sufficiently high at this concentration or the microorganisms multiplied rapidly after initiation of cultures.

WA amended with unautoclaved coir or peat completely inhibited the growth of *F. solani*, but allowed the growth of various microorganisms. On the other hand, WA amended with autoclaved coir, peat, or unautoclaved malt extract allowed the growth of *F. solani* and microorganisms did not grow on these media, implying that microorganisms played a significant role in suppressing the growth of *F. solani* on WA amended with unautoclaved coir or peat.

A. terreus was consistently isolated from coir and strongly inhibited mycelial growth of soilborne plant pathogens, especially *Phytophthora* species. This finding is supported by the work of Almassi et al. (1996), who found that a strain of *A. terreus* isolated from organic mulch inhibited the growth of *P. cinnamomi* and produced an antifungal metabolite, terrecyclic acid A, when grown in liquid culture. In this study, a clear zone of inhibition was observed when *A. terreus* was cultured in the same petri plate with some of the pathogens tested, implying that the inhibition may have been due in part to the production of a diffusible compound.

Isolation of *A. terreus*, *Paecilomyces* sp., and *Trichoderma* sp. from coir in this study is in agreement with a recent study (Waller et al., 2008) in which 29 different species of fungi were identified in 38 substrates, which included six coir substrates. Fungi found in coir included *Trichoderma viride*, *Paecilomyces fumosoroseus*, *Galactomyces geotrichum*, *Eupenicillium*

reticulisporum, and several *Penicillium* species (Waller et al., 2008).

Candole and Evans (2004) demonstrated that when tomato seedlings were grown in coir, the incidence of damping off was reduced by 88%, 90%, and 41% for *P. capsici*, *P. nicotianae*, and *P. aphanidermatum*, respectively, compared with peat. Our study complements the study of Candole and Evans (2004) by demonstrating in vitro that coir can suppress soilborne plant pathogens and that this suppression is largely due to microorganisms associated with the substrate.

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