

# The Effect of a Garlic Extract and Root Substrate on Soilborne Fungal Pathogens

Ramsey Sealy<sup>1,2</sup>, Michael R. Evans<sup>2,3</sup>, and Craig Rothrock<sup>1</sup>

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**SUMMARY.** *Pythium aphanidermatum*, *Pythium irregulare*, *Pythium ultimum*, *Phytophthora cinnomomi*, *Phytophthora nicotianae*, *Rhizoctonia solani*, *Fusarium oxysporum*, and *Thielaviopsis basicoli* grew and eventually covered petri plates containing a nutrient solution alone, but they failed to grow in nutrient solutions containing 10% or higher levels of garlic extract or a fungicide control. When plugs containing the fungal organisms exposed to 10% garlic (*Allium sativum*) extract solution for 48 h were washed and transferred to fresh cornmeal agar (CMA) growth medium, only *F. oxysporum* displayed growth. However, growth of *F. oxysporum* was limited to no greater than 2 mm from the original inoculum plug. After a single application of a solution containing at least 35% garlic extract or two applications containing 25%, viable *P. aphanidermatum* could not be recovered from a peat-based root substrate. By contrast, after a single application of a solution containing 25% garlic extract or two applications of 10%, we were unable to recover viable *P. aphanidermatum* from a sand substrate. When peat treated with increasing concentrations of garlic extract was placed on CMA inoculated with *P. aphanidermatum*, the first visual sign of a zone of inhibition occurred for peat saturated with 30% garlic extract solution and the zone increased as the garlic extract concentration increased. By contrast, when sand treated with increasing concentrations of garlic extract was placed on CMA inoculated with *P. aphanidermatum*, the first visual sign of a zone of inhibition occurred when saturated with 10% garlic extract solution. Therefore, the garlic extract was found to be fungicidal against a broad range of soilborne fungal organisms, but the concentration required to kill the organisms varied depending on root substrate.

Plant loss resulting from root rot-causing fungal pathogens is a significant problem for ornamental and vegetable producers. The most common method of control for these diseases involves the use of chemical fungicides as substrate drench treatments (Hanan, 1998). However, environmental concerns, costs, reentry interval requirements, and the increased interest in organic and biorational production have concomitantly increased the interest in alternatives to traditional synthetic chemical fungicides.

Garlic extracts have been shown to be effective at controlling several foliar fungal pathogens (Lozano et al., 2000; Quarnstrom, 1992; Raghavaiah and Jayaramaiah, 1987;

Saniewska, 1995; Singh et al., 1995). Extracts of garlic have also been demonstrated to have antifungal properties against some soilborne fungi. Russell and Mussa (1977) found that a crude juice extract of crushed garlic cloves inhibited in vitro growth of *Fusarium solani* f.sp. *phaseoli* and provided adequate in vivo control of root rot of 'Seafare' common bean (*Phaseolus vulgaris*) when applied as a seed treatment. Singh et al. (1979) observed that when seeds of gram (*Cicer arietinum*) were treated with an aqueous garlic leaf extract, the resulting seedlings, when placed in soil infested with *Fusarium oxysporum* f.sp. *ciceri* and *Sclerotinia sclerotiorum*, were wilt-free, whereas untreated seeds resulted in seedlings

with wilt symptoms. Singh and Singh (1980) also found that garlic oil suppressed sclerotial formation by *Rhizoctonia solani* and killed the organism when hyphal discs were exposed to the oil in a petri plate assay. They concluded that the inhibition of sclerotia was in part the result of the inhibition of hyphal growth. Singh et al. (1990) found that ajoene, a compound isolated from garlic, inhibited spore formation in several pathogenic fungi in culture. Singh et al. (1992) reported that at higher concentrations, ajoene inhibited mycelial growth, sporangium formation, and zoospore germination of *Phytophthora drechsleri* f.sp. *cajani* in culture. Tariq and Magee (1990) reported that volatile compounds from a crude aqueous extract of garlic inhibited the germination of microconidia and hyphal extension of *F. oxysporum* f.sp. *lycopersici* in culture. Bianchi et al. (1997) conducted ultrastructural studies of hyphae of phytopathogenic fungi treated with micronized garlic powder and observed that the garlic powder strongly inhibited hyphal development with changes in cytoplasm, cell walls, and cell membranes. However, Mercado and Rodriguez (2001) treated two soilborne phytopathogens (*R. solani* and *Myrothecium rostratum*) with a garlic compound called "Garlic Barrier" and found little effectiveness in reducing populations of either pathogen in culture.

The objectives of this study were to determine the effect of a commercially available garlic extract on common soilborne fungal pathogens in vitro and to determine how root substrate affects efficacy.

## Materials and methods

EFFECT OF GARLIC EXTRACT ON SOILBORNE FUNGAL PATHOGENS IN AN IN VITRO NUTRIENT SOLUTION SYSTEM. Isolates of *Pythium aphanidermatum*, *Pythium irregulare*, *Pythium*

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<sup>1</sup>Department of Horticulture, University of Arkansas, 315 Plant Science Building, Fayetteville, AR 72701

<sup>2</sup>Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701

<sup>3</sup>Corresponding author. E-mail: mrevas@uark.edu.

## Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.1013	atmosphere(s)	MPa	9.8692
29.5735	fl oz	mL	0.0338
2.5400	inch(es)	cm	0.3937
25.4000	inch(es)	mm	0.0394
1	meq·100 g	cmol·kg <sup>-1</sup>	1
(°F - 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

*ultimum*, *Phytophthora cinnomomi*, *Phytophthora nicotianae*, *Rhizoctonia solani*, and *F. oxysporum* were grown on reconstituted BBL cornmeal agar (Becton, Dickinson and Co., Sparks, Md.) in 15-cm-diameter petri plates in the dark at 20 to 22 °C for 7 d. *Thielaviopsis basicola* was cultivated on TB-CEN media (Specht and Griffin, 1985) for 21 d. After the culture period, 5-mm-diameter plugs of growth medium containing the pathogens were placed into sterile 15-cm-diameter petri dishes containing 10 mL of 60% clarified V-8 broth nutrient solution (Erwin and Ribeiro, 1996) with or without the addition of a commercial fungicide or garlic extract (Garlic GP Ltd, Co., San Antonio, Texas). All solutions contained 60% clarified V-8 nutrient broth; the remaining 40% of the solution was sterile deionized water, fungicide, or garlic extract solution. Metalaxyl (Subdue Maxx; The Scotts Co., Marysville, Ohio) was used as the fungicide treatment for the *Pythium* and *Phytophthora* species, PCNB (Terraclor; Chemtura, Middlebury, Conn.) was used as the fungicide treatment for *R. solani* and *F. oxysporum*, and triadimenol in combination with thiram (RTU-Baytan-Thiram; Gustafson LLC, Plano, Texas) was used as the fungicide treatment for *Thielaviopsis basicola*. All fungicides were used at the rates recommended by the manufacturers. Concentrations of garlic extract used were 0%, 10%, 15%, 20%, 25%, and 30% (v/v of total nutrient solution) of the product in sterile deionized water. Cultures were placed in a dark incubator at 20 ± 2 °C. Growth was measured daily over an 8-d period beginning after 2 d. Two plates of each treatment were conducted for each pathogen and the experiment was repeated three times (blocked over time). For each replication, one of the discs grown in solution containing 10% garlic solution was removed after 48 h, washed twice in sterile deionized water, placed on CMA, and incubated as described for 3 d to determine if regrowth of the fungal pathogens would occur when removed from the garlic solution. Growth was recorded after 3 d. An analysis of variance was conducted to determine if significant differences in fungal growth occurred among the treatments. When significant differences occurred, a least significant

difference (LSD) mean separation test ( $\alpha = 0.05$ ) was conducted to determine individual differences among means.

**EFFECT OF GARLIC EXTRACT ON *PYTHIUM APHANIDERMATUM* IN PEAT AND SAND-BASED ROOT SUBSTRATES.** Calcitic lime was added to a commercially obtained sphagnum peat (Sun Gro Horticulture, Bellvue, Wash.) to adjust the initial pH to ≈5.5. An organic root substrate was formulated by blending 80% (v/v) of the amended sphagnum peat and 20% perlite (Sun Gro Horticulture). The peat substrate and screened, washed, and dried sand (Quikrete Co., Atlanta) were sterilized twice by autoclaving at a temperature of 121 °C and 0.131 MPa for 90 min at 24-h intervals.

Oospores of *P. aphanidermatum* were produced by growing the organism on oatmeal agar for 10 d. The oospores were harvested by processing the oatmeal agar plates in a Waring blender (Waring, Torrence, Calif.) with sterile deionized water aseptically. Oospores in the crude (unfiltered) extract were counted using a hemocytometer. The peat and sand-based root substrates were inoculated with 10 mL of the oospore suspension ( $\approx 5.5 \times 10^5$  oospores/mL) per 250 mL of root substrate. The inoculated substrates (250 mL per container) were placed into 8.4-cm-diameter plastic containers (6.9-cm depth and 320-mL volume) and drenched with 100 mL of a solution containing 0% (sterile deionized water) to 50% garlic extract in 5% increments. The volume was selected so as to saturate the substrates. Two containers were used for each treatment. After 3 d, one container of each treatment was drenched a second time with 100 mL of the appropriate garlic extract solution. Three days after the application of the garlic treatments, 3-mL samples of root substrate were taken from the containers ≈2.5 cm below the surface. Samples were placed into 15-cm-diameter petri plates containing P<sub>5</sub>ARP (Jeffers and Martin, 1986) medium to control for viability of the propagules of *P. aphanidermatum*. Because the presence of peat or sand made observations for growth difficult, transfers from the medium-containing plates were made to a second P<sub>5</sub>ARP plate to control for growth. Transfers were accomplished by removing the medium from the

plate and aseptically cutting squares of medium from three different regions of the original plate containing the substrate. These squares were then placed on sterile P<sub>5</sub>ARP medium. The experiment was repeated three times. Data were analyzed using a  $\chi^2$  test to determine if garlic drench concentration significantly affected survivability of propagules of *P. aphanidermatum* in the substrates.

**QUANTIFICATION OF THE LETHAL DOSE OF GARLIC EXTRACT AND THE DEVELOPMENT OF A ZONE OF INHIBITION FROM GARLIC EXTRACT-TREATED PEAT AND SAND-BASED ROOT SUBSTRATES.** The sphagnum peat and sand-based root substrates as described were saturated with a solution containing 0%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, or 60% garlic extract. *Pythium aphanidermatum* was cultured on CMA as described. After 6 d, a plug of medium containing growth of *P. aphanidermatum* was placed on one side of a 15-cm-diameter sterile petri plate containing P<sub>5</sub>ARP medium. A 10-mL sample of each garlic-saturated substrate was placed on the opposite side of the plate. Plates were examined after 3 d. This study was repeated three times with two plates per replication.

Nonlinear regression analysis was performed using GraphPad Prism (version 3.00 for Windows; GraphPad Software, San Diego) to determine the effect of garlic extract concentration on the growth of *P. aphanidermatum*. On plotting the zone of inhibition against garlic concentration, a sigmoidal growth function [ $y = top/1 + 10^{(Con50 - x)/hillslope}$ ] was chosen as the model, where  $y$  was the zone of inhibition,  $top$  was the maximum zone of inhibition,  $Con50$  was the concentration of garlic resulting in half the maximum inhibition, and  $hillslope$  was the relative rate of increase from no zone of inhibition to the  $top$  value. The effect of root substrate type on garlic efficacy was considered significant when the  $top$ ,  $Con50$ , or  $hillslope$  parameters for the peat and sand substrates had nonoverlapping 95% confidence intervals (CIs).

## Results

**EFFECT OF GARLIC EXTRACT ON SOILBORNE FUNGAL PATHOGENS IN AN IN VITRO NUTRIENT SOLUTION SYSTEM.** All *Pythium*, *Phytophthora*, *Rhizoctonia*, *Fusarium*, and *Thielaviopsis*

species grew and eventually covered the petri plates containing nutrient solution alone, but they failed to grow in nutrient solutions containing 10% or higher levels of garlic extract or the fungicide controls (data not shown). When plugs were taken from the petri plates containing nutrient solution with 10% garlic extract, washed with deionized water, and transferred to fresh unamended CMA growth medium, all *Pythium* and *Phytophthora* species tested, *R. solani*, and *T. basicoli* failed to grow out into the growth medium. However, a single replication in one of the repetitions of *F. oxysporum* did grow into the CMA medium, although growth was limited to less than 2 mm from the original inoculum plug. In all other replications, *F. oxysporum* failed to grow when washed and placed on fresh unamended CMA.

**EFFECT OF GARLIC EXTRACT ON *PYTHIUM APHANIDERMATUM* IN PEAT AND SAND-BASED ROOT SUBSTRATES.** After a single application of a solution containing at least 35% garlic extract, we were unable to recover viable *P. aphanidermatum* from the peat-based root substrate (Table 1). When two applications of a garlic extract solution containing at least 15% garlic extract were applied at a 3-d interval, we were unable to recover viable *P. aphanidermatum* from the peat-based root substrate. By contrast, after a single application of a solution containing 25% garlic extract, we were unable to recover viable *P. aphanidermatum* from the sand root substrate. When two applications of a 10% garlic extract solution were applied at 3-d intervals to sand, we were unable to recover viable *P. aphanidermatum*. For both peat and sand substrates, using either one or two applications, garlic extract concentration significantly affected survivability of *P. aphanidermatum* in the root substrate.

**QUANTIFICATION OF THE LETHAL DOSE OF GARLIC EXTRACT AND THE DEVELOPMENT OF A ZONE OF INHIBITION FROM GARLIC EXTRACT-TREATED PEAT AND SAND-BASED ROOT SUBSTRATES.** The first visually apparent and measurable zone of inhibition occurred when the peat substrate saturated with 30% garlic extract was placed on a P<sub>5</sub>ARP growth medium-containing plate

opposite of a plug of *P. aphanidermatum*. At 30% garlic extract, the zone of inhibition was ≈0.07 cm (Fig. 1). As the concentration of garlic extract increased to 60%, the zone of inhibition increased to ≈1.8 cm following the model  $y = 1.934 / (1 + 10^{[(46.05 - X) \times 0.097]})$  where  $y$  was the width of the zone of inhibition and  $X$  was the concentration of garlic extract.

In sand, the first visually apparent and measurable zone of inhibition occurred when 10% garlic extract was placed on a P<sub>5</sub>ARP growth medium-containing plate opposite of a plug of *P. aphanidermatum*. At 10% garlic extract, the zone of inhibition was ≈0.2 cm (Fig. 1). As the concentration of garlic extract increased to 60%, the zone of inhibition increased to ≈1.7 cm following the model  $y = 1.739 / (1 + 10^{[(26.98 - X) \times 0.051]})$  where  $y$  was the width of the zone of inhibition and  $X$  was the concentration of garlic extract.

The *Con50* was significantly higher for peat (95% CI = 44.7 to 47.4) than for sand (95% CI = 24.9 to 29.0). Therefore, a higher concentration of garlic was required to reach half the maximum level of inhibition when applied to peat than when applied to sand. The *hillslope* was also significantly higher for peat (95% CI = 0.08 to 0.12) than for sand (95% CI = 0.04 to 0.06). Therefore, although a higher concentration of garlic was required to initiate a zone of inhibition when applied to peat, as the concentration of garlic applied to peat increased, the zone of inhibition

increased at a greater rate than that for sand and by a garlic concentration of ≈50%, no significant difference in the zone of inhibition occurred and the *top* parameter was not significantly different between peat and sand.

## Discussion

In the in vitro nutrient solution studies, growth of all fungal organisms tested was inhibited at the lowest concentration (10%; v/v) of garlic extract tested. Therefore, the a.i.(s) in the garlic extract were able to inhibit growth of a wide range of soilborne fungal organisms. Garlic extracts have been shown to contain at least several biologically active compounds and this diversity of compounds may account for the garlic extract's ability to affect a wide range of soilborne fungal pathogens (Avato et al., 2000; Kshemkalyani et al., 1990; Kyung and Lee, 2001; Naganawa et al., 1996; Reimers et al., 1993; Singh et al., 1992; Tariq and Magee, 1990). With the transfer of mycelial discs exposed to the 10% garlic extract to fresh untreated growth medium, none of the organisms exhibited growth on CMA plates with the exception of a single replication of *F. oxysporum*. The failure of the organisms to develop when washed and transferred to fresh CMA medium supports the conclusion that the organisms were killed by the garlic extract and thus the garlic extract was fungicidal as opposed to being fungistatic. The limited mycelial growth from the single

**Table 1. Growth of *Pythium aphanidermatum* from peat-based and sand root substrates drenched with different concentrations of garlic extract<sup>a</sup>.**

Garlic extract concn (%)	Sphagnum peat substrate		Sand substrate	
	Single drench	Double drench	Single drench	Double drench
0	+	+	+	+
5	+	+	+	+
10	+	+	+	-
15	+	-	+	-
20	+	-	+	-
25	+	-	-	-
30	+	-	-	-
35	-	-	-	-
40	-	-	-	-
45	-	-	-	-
50	-	-	-	-
Significance	*	*	*	*
$\chi^2$ value	33.6	35.7	32.7	28.8

<sup>a</sup> "+" is used to indicate treatments in which *P. aphanidermatum* was recovered from the substrate, whereas "-" is used to indicate when the organism was not recovered from the substrate.

\*Significant at the 0.001 level.

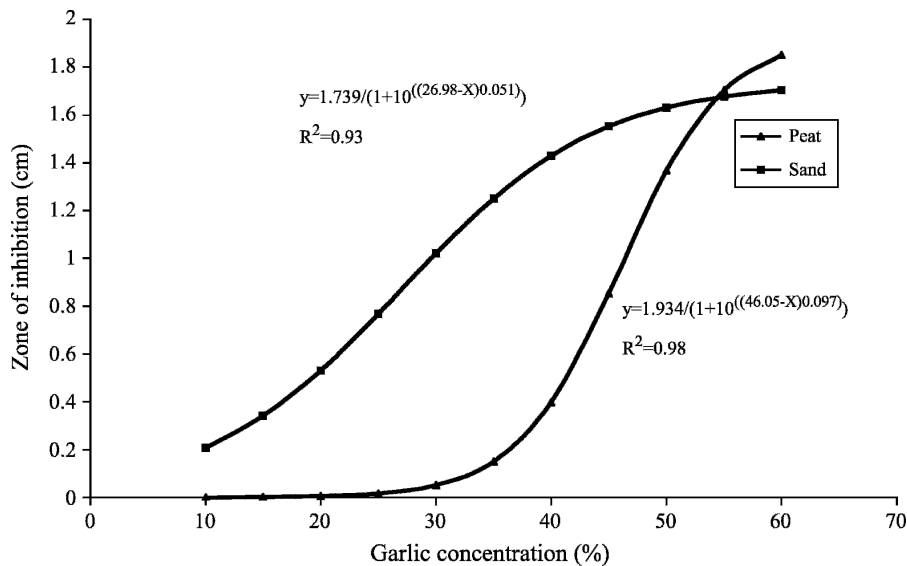


Fig. 1. The zone of inhibition of growth of *Pythium aphanidermatum* created by sphagnum peat and sand saturated with increasing garlic extract concentrations. The model was  $[y = \text{top} / (1 + 10^{((\text{Con50}-x)/\text{hillslope}))}]$  where  $y$  was the zone of inhibition,  $\text{top}$  was the maximum zone of inhibition,  $\text{Con50}$  was the concentration of garlic resulting in half the maximum inhibition, and  $\text{hillslope}$  was the relative rate of increase from no zone of inhibition to the  $\text{top}$  value (1 cm = 0.3937 inch).

*F. oxysporum* replication may also have been a data artifact resulting from experimental error or a 10% garlic solution may have been on the lower range of the effective concentration required to kill *F. oxysporum*, and a population of the organism in one of the CMA plugs may have escaped the full effect of the garlic extract.

When the peat-based substrate was drenched with a solution containing at least 35% garlic extract, we were unable to recover viable *P. aphanidermatum*. Presumably, this was because the organism was killed by the garlic extract. The concentration required to kill the organisms in the peat-based substrate was reduced to 15% when two substrate drench applications were made. Therefore, a significantly higher concentration of garlic extract was required to kill the organism in peat than in the in vitro nutrient solution system. Furthermore, the effect of the garlic extract was cumulative so that with a repeat application, the concentration could be reduced. When drenched into a sand substrate, the concentration required to kill the organism decreased to 25% for a single application and 10% for two applications. Like with peat, the effect of the garlic appeared to be cumulative, but the

concentrations required to kill the organism in sand were significantly lower than for peat.

When sphagnum peat saturated with varying concentrations of garlic extract was placed on CMA growth medium opposite of *P. aphanidermatum*, the first visible zone of inhibition occurred at a garlic extract concentration of 30%. This again is higher than that required in the first in vitro nutrient solution system. This was even though the pathogen was growing in an in vitro nutrient solution system on CMA medium. Thus, because a 10% garlic extract solution was effective at killing *P. aphanidermatum*, the pathogen must not have been exposed to that concentration (a.i.) until the peat had been drenched with at least 30% garlic extract. Because a higher concentration of garlic was not required for sand, the sphagnum peat may have in some way been adsorbed or inactivated the active component(s) in the garlic extract.

One of the major differences between peat and sand was the cation exchange capacity (CEC). A typical sphagnum peat may have a CEC of 100 to 120 meq per 100 g, whereas sand would typically be 2 to 5 meq per 100 g (Hanan, 1998). One

possible explanation for the difference in the effective concentrations of garlic extract between peat and sand may have been the difference in the CEC between the two substrates and thus their ability to adsorb and potentially inactivate the active components of the garlic extract. Such a situation has been reported for plant growth regulators applied as root substrate drenches for height control of greenhouse crops. Application guides often recommend that when growth regulators are applied to root substrates containing composted bark that the concentration of the growth regulator be increased as the a.i. of the growth retardant is by some means inactivated by the composted bark (Barrett, 1982). Additionally, soil organic matter has been demonstrated to adsorb and absorb some herbicides and thereby reduce their efficacy (Minogue et al., 1988; Reynolds et al., 1993; Rytwo and Tavasi, 2003; Said et al., 2004). A similar situation might occur for the active component(s) of garlic when applied to sphagnum peat.

Although the garlic extract was fungicidal against a wide range of soilborne fungal pathogens, the effective concentration varied depending on the number of applications and the root substrate type. A number of different types of garlic extracts have been shown to be effective at controlling fungal pathogens. These products vary from crude garlic extract (Russell and Mussa, 1977), garlic oil (Singh and Singh, 1980), a compound extracted from garlic (Singh et al., 1990) to the product used in this study. Although the garlic extract used in this study was fungicidal against a wide range of soilborne fungal pathogens, the effective concentration varied depending on the number of applications and the root substrate. Therefore, grower recommendations on the effective use of garlic extract to control soilborne fungal pathogens must take into account the root substrate or soil type to which the treatment is made. In some cases, very high concentrations of garlic may be required such as occurred in peat in these studies. These high concentrations might be uneconomic. Furthermore, high concentrations might be phytotoxic to some plant species that may require growers to experiment when using

garlic extract to determine efficacy and potential phytotoxicity under their specific conditions.

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