Effect of a Garlic Extract on Growth of Select Soil-borne Fungal Organisms in Culture

Intermediate Report

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I. Objectives

- To determine the efficacy of a garlic extract for inhibiting growth of the soil-borne fungal plant pathogens *Pythium ultimum, Pythium irregular, Pythium aphanidermatum, Phytophthora nicotiana, Phytophthora cinnamomi, Phytophthora capsici, Rhizoctonia solani, Thielaviopsis basicola,* and *Fusarium oxysporum* when grown in culture.

- To determine concentration effects on the efficacy of a garlic extract for inhibiting growth the soil-borne fungal plant pathogens listed above.

II. General Methods and Procedures

The efficacy of the garlic extract was tested against nine (9) soil-borne fungi that cause root and lower stem rots of plants. The fungal pathogens included: *Pythium aphanidermatum, Pythium irregular, Pythium ultimum, Phytophthora capsici, Phytophthora cinnamomi, Phytophthora nicotiana, Rhizoctonia solani, Fusarium oxysporum* f.sp. *lycopersici, Fusarium oxysporum* f.sp. *spinaciae*, and *Thielaviopsis basicola*. All isolates used in this study were known to be pathogenic to various cultivated plant species.

All isolates of *Pythium aphanidermatum, Pythium irregular, Pythum ultimum, Phytophthora capsici, Phytophthora cinnamomi, Phytophthora nicotiana, Rhizoctonia solani, Fusarium oxysporum* f.sp. *lycopersici, Fusarium oxysporum* f.sp. *spinaciae* were grown on standard corn meal agar (CMA) for 8 to 9 days. Plugs from inoculum plates were taken using a #4 cork borer (5 mm diameter). Inoculum plugs were placed into 5-cm sterile plastic petri dishes containing 10 ml of a basal nutrient solution that consisted of 60% (v/v) clarified V-8 juice and sterile distilled water (SDW). Basal nutrient solutions were amended with the appropriate treatments. Treatments included SDW (non-treated control), a fungicidal agent appropriate to each species (metylaxyl for the *Pythium* and *Phytophthora* species, PCNB for *Rhizoctonia*, and Allegiance® for *Fusarium*), or 10%, 15%, 20%, 25%, or 30% (v/v) garlic extract. Cultures were placed into a growth

chamber and maintained in the dark at 22°C±1°C. After 48 hours, mycelial growth (if any was visible) from culture plugs was measured.

Additionally, plugs were taken from one of the two replicate treatments for each fungal isolate for the non-treated control and the 10% garlic extract treatments, washed in SDW and placed on CMA plates to check for viability of the inoculum after 48 hours exposure to the treatments. Plates were allowed to remain in the growth chamber for eight (8) days. After eight (8) days, any additional growth from the inoculum plug was measured. All treatments were replicated twice and three repetitions of the experiments were performed.

For *Thielaviopsis basicola*, chlamydospores, rather than mycelial growth, were challenged with the treatments. Treatments were as listed above. However, 0.05-ml of a stock solution of chlamydospores were placed in sterile capped centrifuge tubes with 1-ml of the appropriate treatment. The fungicide control for *Thielaviopsis* basicola was Baytan®. After 24 hours, tubes were spun down for 10 minutes at 13,000 rpm. A 0.1-ml sample of the concentrate was spot plated onto TBCEN agar (selective for *Thielaviopsis basicola* and allows for growth of this obligate parasitic fungus) in duplicate. Concentrates were then washed twice in SDW, being agitated and concentrated as described above. These washed concentrates were plated as described above to determine if the treatment resulted in death of the *Thielaviopsis basicola* chlamydospores. Each treatment was replicated twice.

III. Results and Discussion

All *Pythium* and *Phytophthora* species tested grew on the untreated basal nutrient solution (Table 1). However, none of the *Pythium* and *Phytophthora* species placed on basal nutrient solution amended with metalaxyl or garlic extract at 10% to 30% grew from the inoculum plugs.

The *Rhizoctonia* species tested grew on the untreated basal nutrient solution (Table 1). In the first replication, *Rhizoctonia* grown on the basal nutrient solution amended with PCNB displayed a limited amount of growth due to an inappropriate PCMB concentration. However, in the second replication, *Rhizoctonia* did not grow on the basal nutrient solution amended with PCNB. *Rhizoctonia* placed on the basal nutrient solution amended with garlic extract at 10% to 30% did not grow from the inoculum plugs.

All *Fusarium* species tested grew on the untreated basal nutrient solution (Table 1). However, none of the *Fusarium* species placed on basal nutrient solution amended with Allegiance® fungicide or garlic extract at 10% to 30% grew from the inoculum plugs.

For all *Pythium*, *Phytophthora*, *Rhizoctonia* and *Fusarium* species tested, when plugs were removed from the treatment solutions, washed and transferred to CMA, all untreated controls grew from the plugs onto the CMA within 48 hours. However, none of those grown in basal nutrient solution amended with 10% garlic extract grew from the plugs after eight (8) days.

Therefore, garlic extract as low as 10% (v/v) inhibited mycelial growth of all of the fungal pathogens tested in this study. Additionally, even when removed from the presence of the garlic solution, fungal pathogens failed to grow and no sign of viable fungal mycelium could be observed. Thus, the garlic extract was not only fungistatic, but also fungicidal.

Research on the efficacy of the garlic extract on *Thielaviopsis basicola* is continuing. We are currently adjusting the methodology used to produce and isolate chlamydospores in order to have a high enough level of these structures produced to conduct reliable tests. We will provide an amended report when the results are completed

Table 1. Growth of fungal species treated with fungicides or varying concentrations of garlic extract.

Fungal pathogen	Treatment[z]	Plates with visible mycelial growth (%)	Measured growth (cm)[y]
Pythium aphanidermatum			
	Fungicide	0	0
	Non-treated control	100	2.0
	10% garlic	0	0
	15% garlic	0	0
	20% garlic	0	0
	25% garlic	0	0
	30% garlic	0	0
Significance		***	***
Pythium irregular			
	Fungicide	0	0
	No treatment control	100	2.0
	10% garlic	0	0
	15% garlic	0	0
	20% garlic	0	0
	25% garlic	0	0
	30% garlic	0	0
Significance		***	***
Pythium ultimum			
	Fungicide	0	0
	No treatment control	100	2.0
	10% garlic	0	0
	15% garlic	0	0
	20% garlic	0	0
	25% garlic	0	0
	30% garlic	0	0
Significance		***	***
Phytophthora capsici			
	Fungicide	0	0
	Non-treated control	100	0.6
	10% garlic	0	0
	15% garlic	0	0
	20% garlic	0	0
	25% garlic	0	0
	30% garlic	0	0
Significance		***	***

Phytophthora cinnamomi			
	Fungicide	0	0
	Non-treated control	100	0.9
	10% garlic	0	0
	15% garlic	0	0
	20% garlic	0	0
	25% garlic	0	0
	30% garlic	0	0
Significance		***	***
Phytophthora nicotiana			
	Fungicide	0	0
	Non-treated control	100	1.4
	10% garlic	0	0
	15% garlic	0	0
	20% garlic	0	0
	25% garlic	0	0
	30% garlic	0	0
Significance		***	***
Rhizoctonia solani			
	Fungicide	50[x]	0.3
	Non-treated control	100	1.7
	10% garlic	0	0
	15% garlic	0	0
	20% garlic	0	0
	25% garlic	0	0
	30% garlic	0	0
Significance		***	***
***Fusarium oxysporum* f.sp. lycopersici**			
	Fungicide	0	0
	Non-treated control	100	1.1
	10% garlic	0	0
	15% garlic	0	0
	20% garlic	0	0
	25% garlic	0	0
	30% garlic	0	0
Significance		***	***

***Fusarium oxysporum* f.sp. spinaciae**			
	Fungicide	0	0
	Non-treated control	100	1.5
	10% garlic	0	0
	15% garlic	0	0
	20% garlic	0	0
	25% garlic	0	0
	30% garlic	0	0
Significance		***	***

*** Significant at the P>F level of 0.001.

[x] Fungicide controls included Metylaxyl for the *Pythium* and *Phytophthora* species, PCNB for *Rhizoctonia*, Allegiance® for *Fusarium* and Baytan® for *Thielaviopsis*.

[y] Growth (cm) from edge of inoculum plug.

[z] Limited mycelial growth occurred in the first control replication due to inappropriate concentration of PCNB fungicide.