Bioassay of different fractions of *Rhus typhina* leaves with demonstrated antimicrobial activity against *Phytophthora sojae*

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Figure 1. *Rhus typhina*

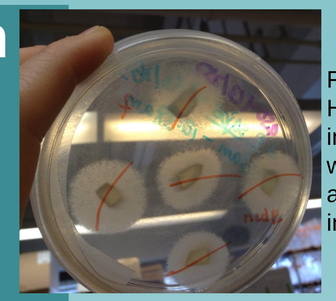

Figure 3. A H-2 plate inoculated with *P. sojae* after 72-hr incubation.

Abstract

R. Typhina leaf extracts have been demonstrated to have antimicrobial activity against *P. sojae* using three solvents, by which *R. Typhina* leaves were partitioned into non-polar, medium polar, and polar compounds. Each partition was mixed with V8 media and tested for its effect on growth of *P. sojae*. The polar and medium polar partitions were most active, although significantly less effective than the commercial fungicide. Through further fractionation of *R. Typhina* leaves and testing with a wider range of concentrations, the active compound(s) might be identified and be used as a natural fungicide.

Introduction

Background:
Plants produce an array of compounds, some of which have antimicrobial activities. In earlier research, extracts from 265 plants parts of 130 native species in the Upper Mississippi River and Red River Basins were tested against four microorganisms. Extracts of 80 inhibited as least one microorganism. Extracts of *Rhus typhina* leaves and seeds showed significant inhibition of the growth of some soil borne pathogens, including *Phytophthora sojae,* a common soil borne pathogen that causes soybean root rot.

Objectives:
•Verify that *R. typhina* leaves have antimicrobial activity against *P. sojae*.
•Determine its relative activity in comparison to that of a commercial fungicide – metalaxyl.
•Identify the most effective solvent for extracting the active compound(s) in *R. Typhina* leaves.
•Investigate the effect of concentration of different leaf partitions, if any, on its antimicrobial activity.

Methods

R. typhina leaf extracts were fractionated into non-polar, medium polar, and polar compounds with solvents hexane, dichloromethane (DCM), and water, respectively, according to “like dissolves like” theory. Each partition was dissolved into solution using 100% methanol (1 µg partition:1 µL methanol).

Three different concentrations - 10, 6, and 2 µg/ml - of each partition were obtained by mixing with autoclaved V8 media – growth media for *P. sojae*, and poured into Petri Dishes. Three control treatments including 6 µg/ml metalaxyl, 10 µl/ml methanol, and V8 media only, were also prepared. Each concentration of each partition and each control was replicated three times.

Cultures of *P. sojae* (mn 25) were grown for 13 days on V8 media. Five 3-mm blocks of these *P. sojae* cultures were inoculated onto each medium plate.

After 72-hour incubation, colony diameter of each block was measured; the percent inhibition was calculated as a percentage of the V8-only control. Figure 3 shows a sample plate ready for measurement.

Results

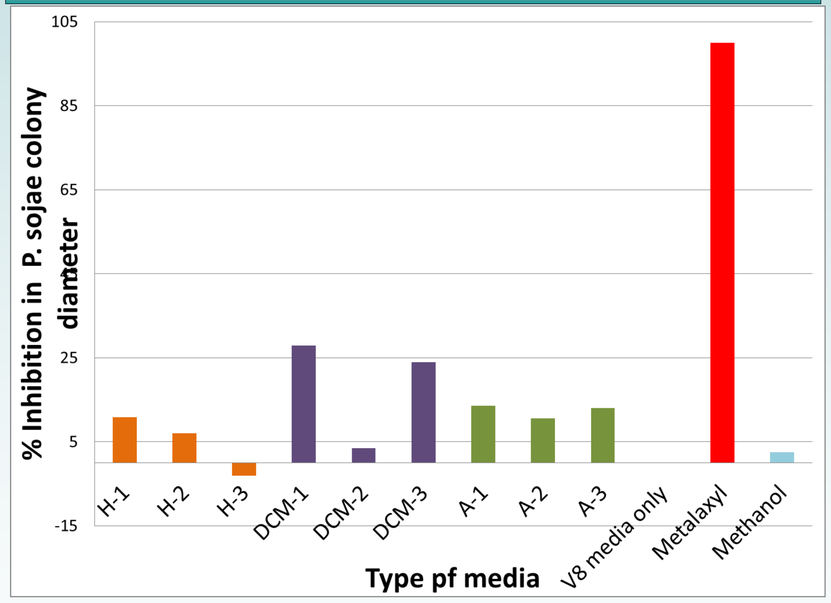

Figure 2. Percent inhibition in colony diameter of *P. sojae* caused by different concentrations of various extracts of *R. typhina* leaves and by controls.
In this experiment, 13-day-old *P. sojae* were inoculated onto various media. H-1, H-2, and H-3 are10, 6, and 2 µg/ml of hexane partition mixed V8 media. DCM-1, DCM-2, DCM-3 are these concentrations of DCM partition mixed V8 media, A-1, A-2, and A-3 are these concentrations of aqueous partition mixed V8 media. Control-media (V8 media only, 6 µg/ml metalaxyl-media,10 µl/ml methanol-media) are presented for comparison.

The results showed that 1) all 3 partitions of *R. Typhina* leaves inhibited *P. sojae* growth when compared to the fraction-free controls; 2) activities of all three fractions were significantly lower than that of metalaxyl; 3) different partitions demonstrated different abilities to inhibit growth of *P. sojae* (P(three partitions have the same effectiveness)= 0.0966); 4) DCM partitions were the most inhibitory, aqueous partitions the second, and hexane the third; 5) concentration did not appear to affect activity in aqueous partition, while activity slightly decreased as concentration increased for hexane and DCM partitions.

Conclusions

R. Typhina leaves demonstrated antimicrobial activities against *P. sojae,* although they were less significant than that of metalaxyl. The active compound(s) are most likely to be polar or medium polar compounds, best extracted by similar polarity solvents like water and dichloromethane, which corresponds with results from earlier studies. In these studies, DCM and aqueous partitions of *R. Typhina* leaves were active, but hexane partition was not against yeast *Candida albicans*. The concentrations of the first two required to complete kill were determined.
Our results suggest that further research should be conducted using higher rates and wider ranges of concentrations of each partition to better understand the effect of concentration. DCM and aqueous partitions can be further fractionated to localize the active compound(s) using methods like flash column chromatography and HPLC-DAD, while UPLC-ESI-MS can be used to aid in elucidating the structure(s) of the active compound(s).

References:
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Gillitzer P, Martin AC, Kantar M, Kauppi K, Dahlberg S, Lis D, Kurle J, Sheaffer C, Wyse D (2012) Optimization of screening of native and naturalized plants from Minnesota for antimicrobial activity. *Journal of Medicinal Plants Research*, 6(6): 938-949.
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