

Pic.1. *Phytophthora sojae* Zoospore,
<http://www.plosbiology.org/article/doi/10.1371/journal.pbio.0020213#>

Analysis of Natural Antimicrobial Extracts of *Rhus typhina* Found in Minneapolis

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Pic.2. Zoospore release from sporangium,
<http://www.apsnet.org/publications/imageresources/Pages/1W000025a.aspx>

Introduction

Plants produce numerous organic compounds that have antimicrobial activity. (Borchardt, 2008b; Gillitzer et al. in review) These natural products are found in tissues and structures throughout the plant, including the stems, berries, mature/young leaves, and roots. The many native and naturalized plant species that grow throughout Minnesota are a potential source of chemicals with antimicrobial properties that could be useful in medicines, cosmetics, preservatives or pesticides. Identification of antimicrobial compounds from a wide array of plant tissues and numerous plant species requires screening of numerous extracts from many different plant sources for their biological activity. Alamar Blue (AB), a nontoxic, water soluble dye, changes color from indigo blue to fluorescent pink in the presence of living cells. This color change is an accurate indicator of the fungistatic activity of plant extracts, via respiration, dissolved in a solution of water, nutrient broth, and AB. *Phytophthora sojae* is a soil borne plant pathogen and major cause of soybean crop loss. Zoospores are a motile reproductive stage of *P. sojae* critical to the infection process.

Objectives

- Develop a simple rapid method of screening plant extracts for fungicidal activity.
- Determine if extracts from a variety of plant tissues and structures differ in their fungicidal activity.

Materials and Methods

Develop and Test Spectrophotometric Viability Assay

- Develop V-8 broth as liquid medium to support *Phytophthora sojae* cultures
- Optimize zoospore production on Chen Zentmeyer Salt Solution
- Assay antifungal activity of commercial fungicides by change in absorbency of AB over 24 hour period.
- All of the data was collected using a Turner SP-830 Photospectrometer

Fungicide effects on fungal respiration

- Figures 1 and 2 display the effect of three fungicides, PCNB, Metalaxyl, and Pimaricin, on the absorbance of the Alamar Blue solution.
- High absorbencies (blue) indicate less respiration
- Low absorbencies (pink) indicate more respiration.
- The absorbance of all different concentrations of PCNB, Metalaxyl, and Pimaricin decreased from higher absorbency to lower absorbency during the 24 hour period of the experiment.

Results



Fig.1. Sample cuvettes containing differing concentrations of PCNB in solution with V8 broth, *P.sojae* zoospores, and Alamar Blue. (From left to right) Cuvettes contain 500, 250, 125, and 0 ug/ml of PCNB.

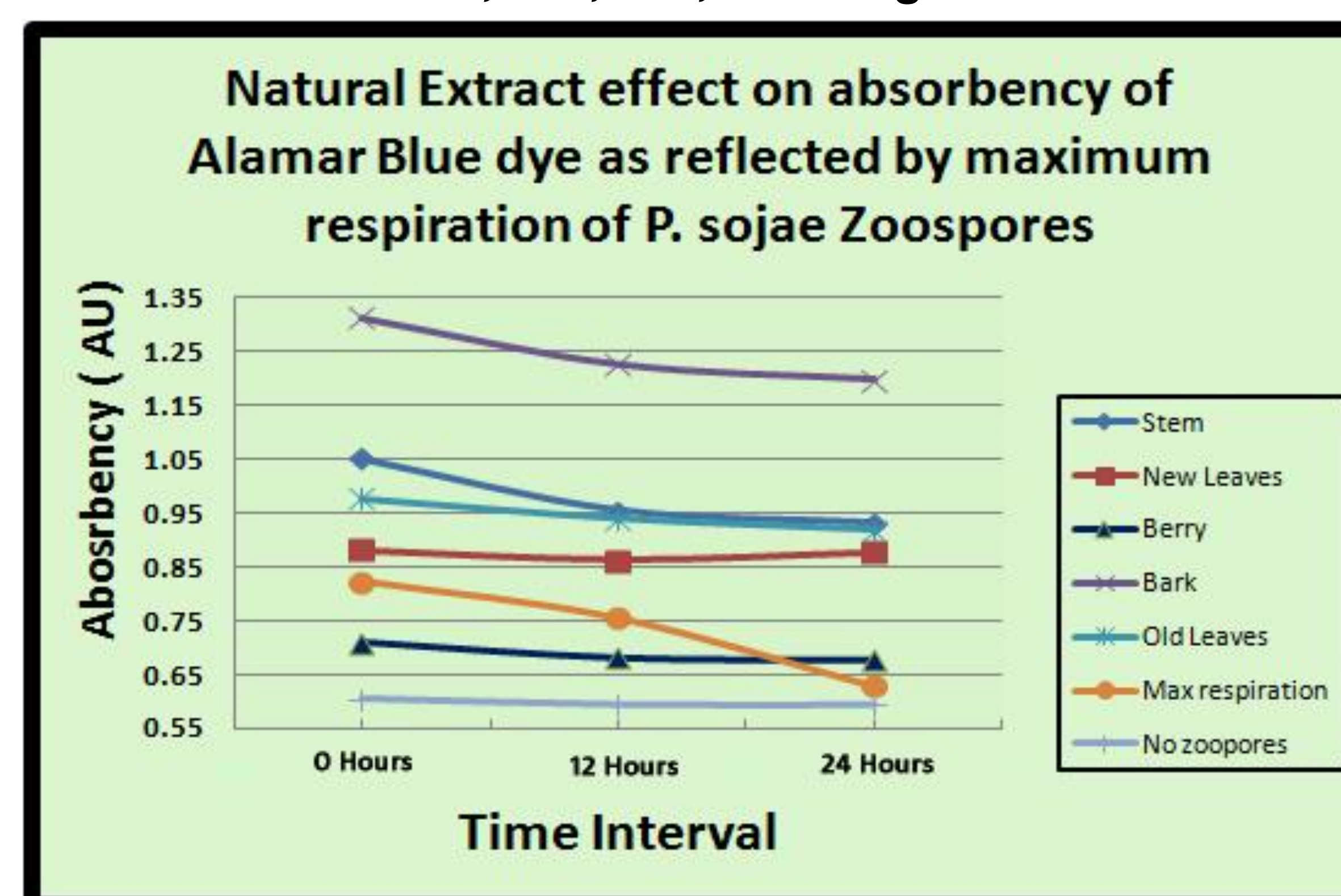


Fig.2. Effect of five natural extracts on absorbency of Alamar Blue. Increasing absorbance is an indication of decreasing fungal respiration. New Leaves and Berry extracts were the most effective at controlling respiration of *P.sojae*.

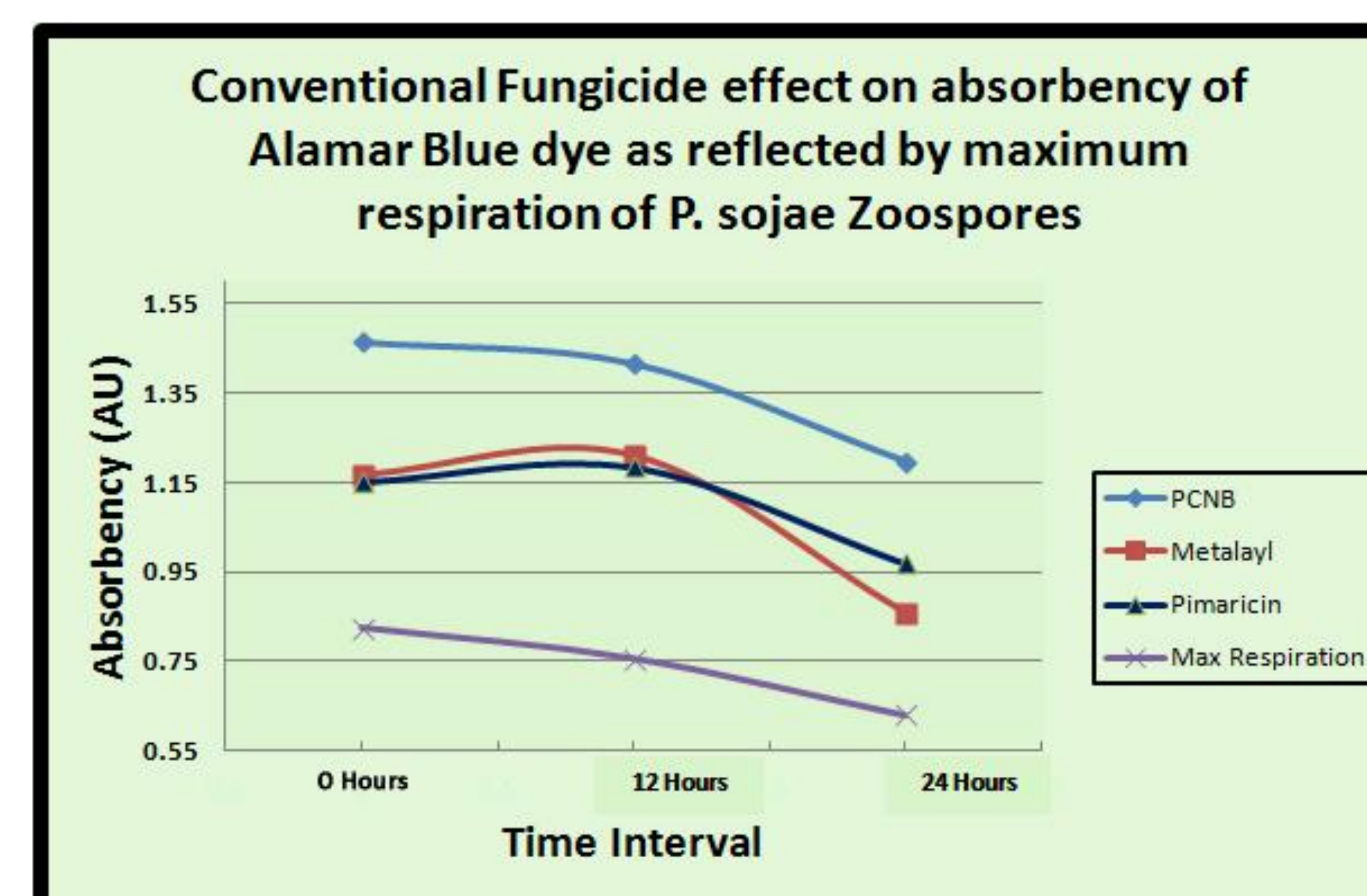


Fig.3. Effect of three fungicides on absorbency of Alamar Blue. Decreasing absorbance is an indication of fungal respiration. PCNB was most effective fungicide at controlling respiration of *P.sojae*.

The absorbance of solutions of three fungicides decreased with increasing time. The absorbance of some of the solutions containing plant extracts had different results. Solutions containing Berry and New Leaf extracts had almost no change in absorbency. Differences in the rate of absorbance suggest that the toxicities of the fungicides differ or that differing stages of the fungal life cycle are affected by the compound. Differences between the natural compounds suggest that different locations on a plant have different antifungal capabilities.

Discussion for this project

The Alamar Blue reaction:

- Detects fungal respiration
- Enables rapid analysis of samples, thus resulting in a larger sample size

Our results indicated that Alamar Blue absorbance:

- Is a measure of AB concentration
- Is proportional to live zoospore numbers
- Is reduced by fungal respiration
- Can be used to detect the antifungal activity of compounds

The results indicate that:

- Plant extracts contain naturally occurring compounds with antifungal activity. Further research is necessary to identify these compounds for use as fungicides and determine rates of application as effective as conventional fungicides
- A colorimetric assay provides a rapid, high-throughput method for evaluating the antifungal activity of plant extracts.

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