

Maple leaf tea extract effects on *Microdochium nivale* growth *in vitro*

A maple leaf tea mixture was made by grinding up maple leaves and then soaking them in water while heating the mixture. The mixture was first filtered to remove coarse particles, and then the tea was subsequently filter-sterilized. The sterilized tea extract was added to PDA in a laminar hood, and two different concentrations of the tea extract were made into PDA plates. All plates had the same amount of PDA concentration. Four replicates of each PDA only control, half-strength, and quarter-strength maple leaf extract plates were inoculated with a plug of *Microdochium nivale* and maintained at 66F. Plates were randomized daily to avoid any environmental effects. Three measurements of the diameter of the fungal growth were recorded and then averaged. Growth data were recorded from 3 to 5 days after inoculation before the control plates were fully covered by the fungus. Both maple tea concentrations significantly inhibited the growth of *M. nivale* (Fig. 1). The final fungal growth on control, half-strength, and quarter-strength plates had an average diameter of 5.4 cm, 1.7 cm, and 3.0 cm, respectively. All treatments tested were significantly different from each other.

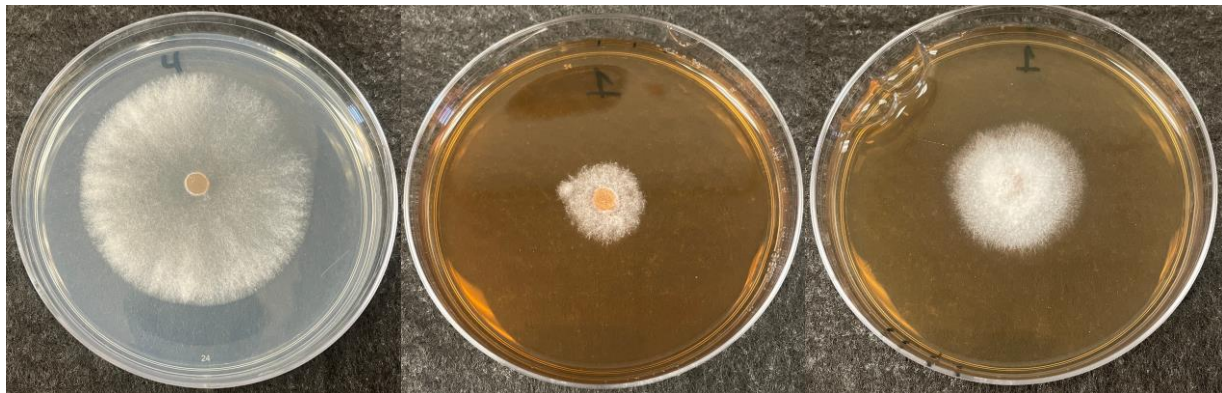


Figure 1. *Microdochium nivale* growth at 5 days after inoculation. From left to right: control PDA, PDA plates amended with half-strength, quarter-strength maple leaf extract.

Fractionating Maple extracts to find active molecules

Crude metabolites were extracted from ground maple leaves using ethyl acetate and subsequently fractionated using a silica column (Figure 2a). Fractions were collected at 2-minute intervals over a 45-minute period. Each fraction was precipitated, resuspended in water containing 0.5% DMSO, and then screened for herbicidal activity using *Chenopodium album* seed germination assays. Root lengths were measured for each treatment to assess the effects of the individual fractions on seedling growth.

Fractions 3, 10, and 18 produced the greatest reductions in root length compared with the control (Figure 2b), suggesting that these fractions contained compounds with potential herbicidal activity. These fractions were subsequently analyzed by mass spectrometry for compound identification. In Fraction 3, quinoline was detected at higher-than-expected concentrations, suggesting that it may be one of the compounds responsible for the herbicidal activity observed in maple leaves.

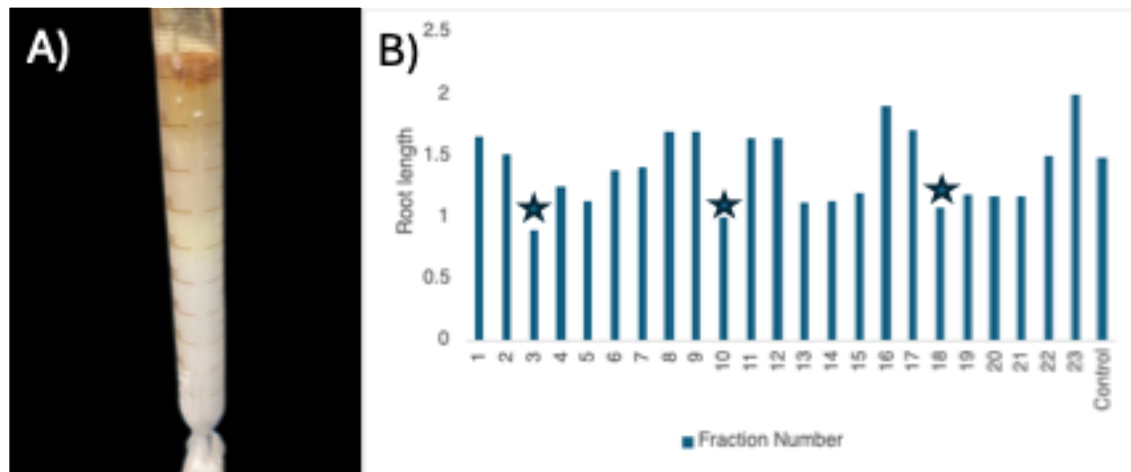


Figure 2. A) An example of the large silica column used for fraction separations. B) Bioactivity of each fraction on root elongation of *Chenopodium album*

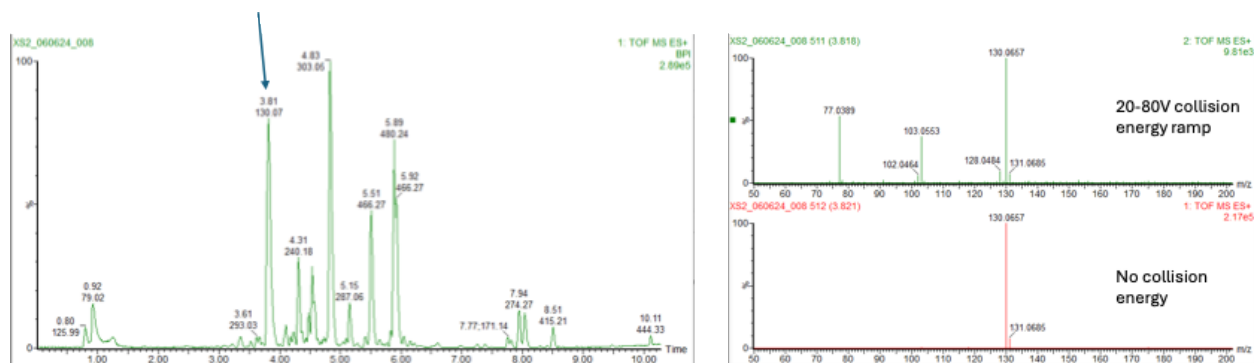


Figure 3. Mass spectra of fraction 3. The compound “quinoline” is highlighted by an arrow. Quoline’s mass fragmentation pattern is also displayed at ~130.065 (expected ~129.16).