Raman Spectroscopic Analysis of Plant Cell Wall in Normal and Diseased Sunflower

Mukesh Roy1, Brian Kontz2, Febina Mathew2, and Anamika Prasad1
1Dept. Of Mechanical Engineering, South Dakota State University, Brookings, SD, USA; 2Dept. of Agronomy, Horticulture and Plant Science, South Dakota State University, Brookings, SD, USA

Statement of Purpose: Plant health and early disease detection are essential for high-yield crop production in agricultural industries. The current approach to plant diagnosis includes chemical analysis and pathophysiological screening. These investigations are time-consuming and require access to specialized plant diagnostic labs. Raman spectroscopic (RS) analysis, on the other hand, can be used to identify biological composition and underlying ultrastructural changes without staining and complicated sample preparation [1]. This work presents investigation and quantification of plant health and growth status (specifically changes in cell-wall) using Raman Spectroscopy (RS). We analyzed the biological composition of sunflower plant at different time points of healthy growth and those affected by a disease called Phomopsis stem canker.

Methods: Dried stems of sunflower plant affected by Phomopsis stem canker disease was collected from field site (Figure 1a). Healthy plants were grown in lab greenhouse and collected at different time points during growth. A 21-day old healthy stem is shown in Figure 1b during healthy growth in the lab-based facility. The samples were stored at room temperature (25ºC). Raman spectroscopic analysis was performed on the stem cross-section using two different approaches. Thick samples were directly kept on the platform for analysis. For thin sections, a water bath-based platform was designed to prevent substrate effect. RS analysis was performed using Bay Spec 2020 spectrophotometer. (BaySpec Inc, USA) fitted with Raman probe (RPB785, InPhotonics Inc, USA) and diode-based 785 nm laser (InPhotonics Inc, USA). Optical microscopy was used to view and guide location for RS (Figure 2). Savitzky-Golay filter of 20 windows sizes were used for smoothing the raw data with periodic boundary condition. A user-defined baseline was carefully fitted for carrying out baseline correction after filter application.

![Figure 1](image1.png)  
**Figure 1:** (a) Diseased sunflower stem cross-section cut longitudinally from root end (S1) towards shoot end (S4) showing medulla in the center with cortex tissue around it, b) Non diseased sunflower stem of 21 days old.

Result and Discussion: The following Raman peaks were observed in the diseased sunflower samples: 790 cm⁻¹, 847 cm⁻¹, 1090 cm⁻¹, and 1330 cm⁻¹ (Figure 3). These peaks correspond to Sucrose, Glucose, Phenylalanine, and Maltose respectively. For non-diseased 21 days old sunflower samples, key Raman peaks at 514 cm⁻¹ corresponding to cysteine was observed (Figure 4).

![Figure 3](image2.png)  
**Figure 3:** Microscopy of diseased sunflower section at the junction of pith and medulla.

![Figure 4](image3.png)  
**Figure 4:** Raman Spectroscopy of non-diseased Sunflower plant at 21 days.

Conclusions: Here, we have characterized healthy and diseases sunflower stem for its compositional alteration and comparison. Overall, we show that RS can be a valuable tool to gain structural and chemical changes in plants under varying physiological conditions (specifically healthy growth and disease) without detailed sample preparation approach.