The Effects of EPS Biopolymer Produced by *Rhizobium Tropici* on Seed Germination, Root Enhancement, and Soil Erosion Resistance

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Abstract:

Soil erosion is a global environmental concern caused by reductions in soil cohesion and water retention. Vegetation with a rich system can be used for erosion control by altering soil properties, such as aggregate stability, hydraulic function, and shear strength [5]. A rich root system can be achieved by utilizing chemical fertilizers. However, the overuse of fertilizers has led to environment contamination by phosphorus (P), nitrogen (N), and potassium (K). The use of biodegradable and environmentally friendly biopolymer-based substrates to enhance root growth has long been a topic for research. Extracellular polysaccharides (EPS) produced from *rhizobium tropici* is a biodegradable biopolymer with an environmentally friendly synthesis process. In this study, the effects of various concentrations of EPS solutions on germination and root growth were examined using two species: green bush beans and rose of sharon. Plants treated with 50 mg/L bulk EPS solution were found to have the highest root weight, the longest root length, and the highest number of roots. In addition, EPS treated green bush bean plants produced fruit after 8 weeks of germination while no fruit was produced in the control group. Metal content analysis was performed by XRF and showed that plants watered with EPS solution contain more K, P, S, and calcium (Ca) than those treated without EPS. The characterizations of EPS powders were determined by XRF analysis, and the results confirm that EPS biopolymer contains a large amount of P, K, Ca, and chloride (Cl) which are beneficial micronutrients for plants. The results indicate that *R. tropici* produced EPS enhances root growth with a strong possibility for erosion resistance.

Keywords: EPS biopolymer, *rhizobium tropici*, root growth, plant growth, soil erosion, biodegradable

1. Introduction

Soil erosion is the loss of the top layer of soil in an area. It can be a result of different erosion agents such as wind, water, animals, or human activities. Loose soil surfaced and detached from the rest of the soil are carried to another location by erosion agent(s), where the particles are dropped off [1]. This leads to the loss of farmland and fertile area, the pollution of loose soil, and sediments in water sources. More severe effects are desertification and more frequent or serious flooding. [2]. Some literature has shown that soil is lost at a rate of 6 Mg ha^{-1} yr^{-1}(Metric ton per hectare per year) or more in the United States alone. Northeastern China experiences as much as 15 Mg ha^{-1} yr^{-1} of soil lost in the region [3]. A solution to this problem is to add more plants to the area where the soil is to be protected. Plants provide a layer of protection due to the initial barrier of stem area, as well as the underground attachment of the root system [4]. The plants also retain water, preventing the top layer of soil from drying out.

The shear force between the top and bottom layers of soil will keep the soil from separating, and the root system's interaction with the soil also provides shear strength. Since roots have high tensile strength, it tightly holds the soil together between the root systems [5]. Studies have shown that plant roots hold soil together to prevent run off, although different plants will have various effectiveness in holding the soil [6]. Lateral growth is considered as well, where the roots are considered as secondary, tertiary, or quaternary roots in the system. Nutrient uptake is not uniform throughout these classifications, leading to the nonuniform growth of plants [6].

One way to increase plant growth is the supply of fertilizers rich in phosphorus, potassium, and nitrogen, which are crucial and beneficial nutrients. However, nutrient pollution may be caused by runoff, leaching, heavy rain, and other factors that may lead to some of these nutrients entering the nearby water stream [7]. Fertilizer pollution has a detrimental effect on the ecosystem, since it affects both the water quality and fish population. Algal bloom is a major result of many of fertilizer pollutants due to a huge burst of nutrients in the water. Algae start growing at a rate faster than usual, which leads to an accumulation of algae, often on the water's surface [8]. These algal blooms are harmful to the environment, especially if the species of algae is toxic. In addition, because algal bloom

floats on the surface of water, it often blocks the sun, which hinders the growth of aquatic plants.

The detrimental effects of fertilizers can be removed with the use of biopolymers. Biopolymers are polymeric substances that are produced by a living organism, consisting of monomer units that are chained together to form a larger molecule. The strain of bacteria used in this study is *Rhizobium tropici* (ATCC strain designation CIAT 899), which produces a gel-like, extracellular polysaccharides (EPS) biopolymers by using glucose, sucrose, or other sugars as a sole carbon source [9]. The energy required to produce EPS from *R. tropici* is significantly less, which makes the synthesis process environmentally friendly, and EPS is a biodegradable biopolymer [10]. Several functions of EPS include surface adhesion, formation of protective barriers, water retention around roots, and the accumulation of nutrients [10]. Those functions suggests that EPS can be used to improve the strength of soil for erosion control and slope stability [11]. Moreover, this specific strain has been studied as an alternative to fertilizer due to its ability to retain water and promote root growth [12]. Since EPS could be composed of multiple polymeric components, alcohol precipitation was used to obtain different fractions. Each fraction has different properties and molecular structures. Therefore, FTIR analysis was used to determine their composition. This study investigates the composition of the EPS, the optimal concentration for plant irrigation, and compares the effectiveness of the different component fractions. The plants studied were green bush beans and rose of sharon and the efficacy of EPS was determined by quantification of seed germination, root growth, branching, and flowers and fruits production for plants watered with regional water and regional water enriched with EPS.

2. Materials and Methods

2.1 Materials

Precipitation of EPS Powders from R. Tropici Biopolymer Solution

In order to investigate the properties and effects of different fractions of biopolymers. Four EPS fractions were precipitated from the biopolymer solution by adding ethanol ratio at different ratio. Four EPS powders (bulk, EPS A, EPS B, EPS C) used in this study were produced and provided by the US Army Corps of Engineers. An illustration of the four EPS powders production from biopolymer solution is shown in Figure 1. A 2L of EPS biopolymers produced from *R. Tropici* was initially a solution, and as starting material. The biopolymer substrates can be separated by adding ethanol into the solutions and isolated through a centrifugation process. Adding ethanol produces a precipitate of the biopolymer due to it being nearly insoluble in ethanol [10]. Ethanol was added to the biopolymer solution with an ethanol ratio of 60%. Then, precipitate was produced and isolated from the solution, and referred as bulk EPS. Consecutive fractionation procedures were performed with different ethanol ratio (listed in Table 1) to obtain the other three EPS fractions, and the procedures were described as below. Ethanol was added to another 2 L of biopolymer solution with an ethanol ratio of 30%, and the isolated precipitate was referred as EPS A. Additional ethanol was added to the supernatants from EPS A until the solution contained 50% ethanol, and that precipitate was labeled as EPS B. The same procedures were repeated by adding more ethanol to reach 65% of supernatants from EPS B and precipitated the fraction that was labeled as EPS C. The isolated precipitate was dried in an oven at 55 °C after each fractionation processes.

Plants Materials

Two types of species, green bush bean and rose of sharon, were used in the germination and potted plant growth experiments.

Table 1. Summary of ethanol ratio of EPS A, EPS B, EPS C.

Sample	Ratio of ethanol to solution
EPS A	30% ethanol and 70% biopolymer solution
EPS B	50% ethanol and 50% supernatants from EPS A
EPS C	65% ethanol and 35% supernatants from EPS B

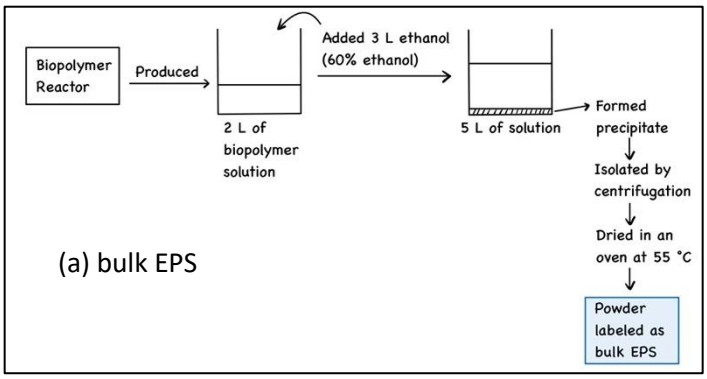

Figure 1. (a) Illustration of EPS powders production from biopolymer solution for bulk EPS.

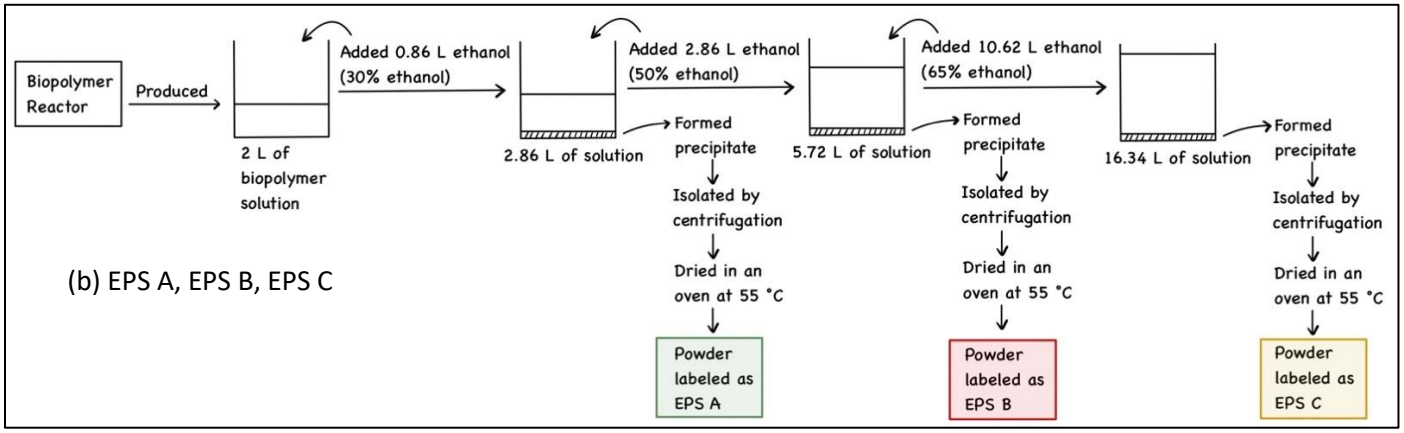

Figure 1. (b) Illustration of EPS powders production from biopolymer solution for EPS fractionations (EPS A, EPS B, EPS C).

2.2 Characterizations EPS Powders

X-ray fluorescence (XRF) spectroscopy was conducted to determine the elemental composition of the EPS powders. The XRF analysis was performed on a Niton XL3t GOLDD+ handheld XRF (Thermo scientific).

Fourier-transform infrared (FTIR) spectroscopy was used to analyze the chemical structures of EPS powders, and it was performed on a Nicolet 560 IR spectrometer with a deuterated friglycine sulfate (DTGS) detector and the data were summed over 256 scans for a better signal to noise ratio.

2.3 EPS Solution Preparation

EPS solution was prepared by dissolving the desired amount of EPS powder into 1 L of tap water. Tap water was used as a solvent in the solution preparation and in the control experiments, rather than DI water. EPS solutions of three different concentration prepared from the bulk EPS powders were used to study the effects of EPS concentration on seed germination and root growth: 25, 50, and 75 mg/L. In addition, four EPS solutions of 200 mg/L concentration were prepared using the four fractions of EPS powders to study the effects of various EPS powders on germination and root development. This concentration was chosen since it represented the lowest value at 50 mg/L and hence would be most sensitive to the differential. The pH of the tap water is 7.5, and all EPS solutions have a pH value of 7.5 $\pm$ 0.3.

2.4 Germination and Growth Condition

All seed germination and plant growth experiments were conducted under growing lights in a greenhouse (Figure 2). The wavelengths of growing lights are between 430 to 470 nm for blue light and between 600 to 800 nm for red light. The temperature was maintained at room temperature, 25 $\pm$ 2 ℃, for seed germination and plant growth.

Figure 2. Greenhouse system with growing lights.

2.5 Green Bush Bean Seed Germination Experiments

In the study of the effects of EPS solution on root growth, 5 green bush bean seeds were covered in paper towels that were saturated by 50 mg/L bulk EPS solution and placed in Petri dishes. As in the control group, seeds were germinated in tap water. In total, three Petri dishes of seeds were germinated per group, and were placed into a larger container as shown in Figure 3. Enough EPS solution or tap water was added in the container to keep the paper towel moist for the germination process. Figure 4 is an illustration of a root system of green bean plant, which shows a general root system consists of a primary root which is from a stem, secondary roots growing from the primary root, tertiary roots growing from the secondary

root. In the root analysis study, weight of the root system, length of primary root and secondary root, and number secondary and tertiary roots were measured and analyzed. The root length was measured using Software ImageJ. A follow-up germination experiment was conducted to examine the various EPS concentrations (25, 50, 75 mg/L of bulk EPS) and fractions of EPS (bulk, EPS A, EPS B, EPS C at 200 mg/L) on bean germination. Table 2 summaries the EPS treatments used in two germination experiments. Weight of the root system and number of roots were measured at the end of experiment for root development analysis.

Table 2. Summary of EPS treatments on germination experiments.

Experiment	Control	Experimental	Germination duration
#1	Tap water	50 mg/L of bulk EPS	13 days
#2	Tap water	25, 50, 75 mg/L of bulk EPS + bulk EPS, EPS A, B, and C at 200 mg/L	9 days

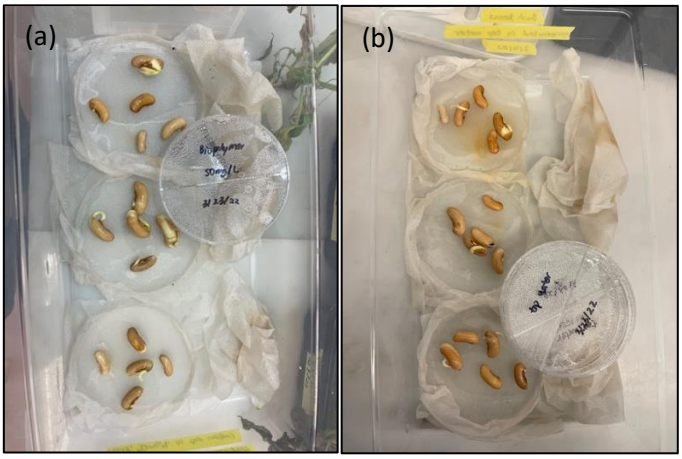

Figure 3. Green bush beans germination in Petri dishes, (a) experimental group – 50 mg/L bulk EPS solution, (b) control group – tap water.

2.6 Potted Plants Experiment

Green bush bean seeds and rose of sharon seeds were germinated using tap water following the procedures described in the seed germination experiments section. After two weeks of germination, seedlings with similar root length were transferred into plant growing trays and grown in potting soil. A picture of rose of sharon plant grew in pot is shown in Figure 5. In the experimental group, plants were watered with concentration of 50 mg/L bulk EPS solution. Plants were watered with tap water in the control group. A soil pH / moisture detector (Sonkir 3-in-1 Soil Moisture Light PH Tester) was used to monitor and record

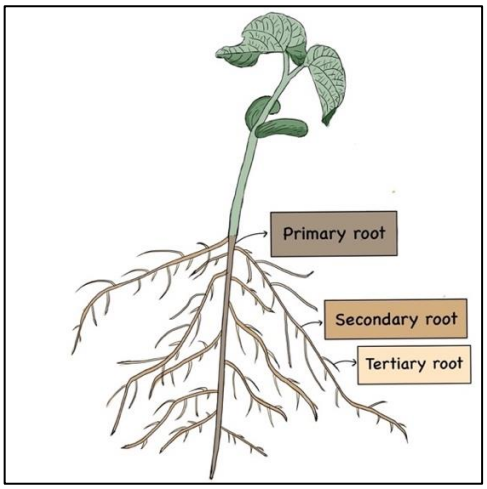

Figure 4. Illustration of a root system of green bean plant.

the pH and moisture level of soil (Figure 6). The soil pH level was maintained at 7.5 ± 0.5 and the soil moisture content was kept at the level of "moist" as indicated on the detector. Rose of Sharon plants were grown in a greenhouse for two months before analysis. The plants were removed from trays to remove soil from roots and the root system were further cleaned through sonication. The weight of Rose of Sharon plants was measured for plant growth analysis. Metal contents analysis in the Rose of Sharon root system was performed by XRF. Green bush bean plants were grown in the greenhouse for 6 weeks before plant analysis. The root system of a bean plant is complex, and it was tangled together with soil. Therefore, it was difficult to remove the soil attached to the bean plant roots even with sonication. XRF analysis was only performed on the green bush bean leaves and fruits for metal detection.

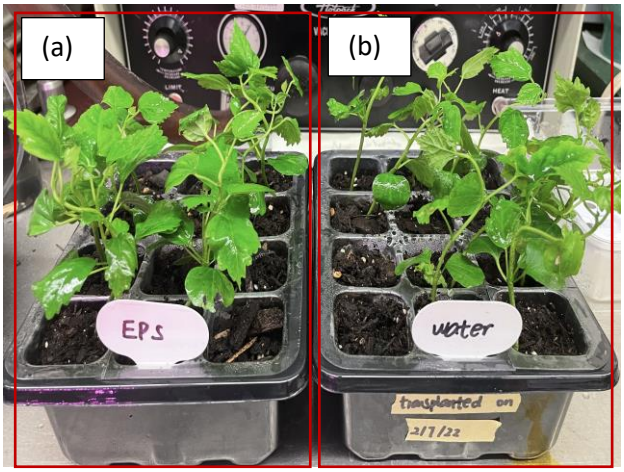

Figure 5. Rose of sharon plants grown in pot (4 weeks after transplantation), (a) watered with 50 mg/L bulk EPS, (b) watered with tap water.

Figure 6. Sonkir 3-in-1 Soil Moisture Light PH Tester.

3. Results

3.1 Effects of 50 mg/L Bulk EPS Solution on Root Growth

In the germination with EPS study, 15 plants in each group were analyzed for root growth. Root weight, length and branches of green bush bean root system was measured and recorded. As shown in Figure 7a, seedlings germinated with 50 mg/L bulk EPS solution have higher root weight with an increase of 16.7% than those seedlings germinated with tap water. Figures 7b and 7c indicate that seedlings treated with EPS solution have longer primary roots and longer secondary roots than those in the control group. The length of the primary root increased by 23.3% and that of secondary roots increased by 17.9%. As shown in Figures 7d and 7c, seedlings germinated with EPS solution have more secondary and tertiary roots than seedlings germinated without EPS. The number of tertiary roots in the experimental group is three times more than that in the control group. A side-by-side comparison of green bush bean root systems is displayed in Figure 8. EPS treated plants have a denser and richer root system than those with no EPS treatment. Overall, green bush beans germinated with EPS solution have higher root weight, longer root length, and more roots. These results indicate that EPS enhances root elongation and root branching for developing a denser root system.

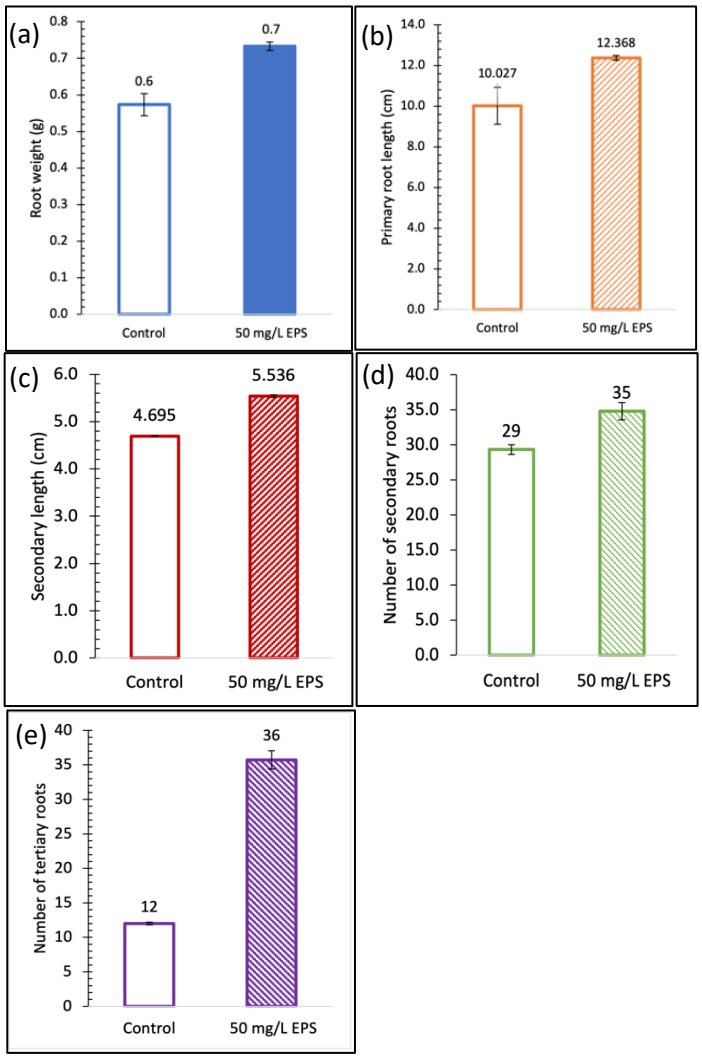

Figure 7. Green bush bean roots analysis results, (a) root system weight, (b) primary root length, (c) secondary root length, (d) number of secondary roots, (e) number of roots.

Figure 8. Side-by-side comparison of green bush bean root systems, (a) germinated with tap water, (b) germinated with 50 mg/L bulk EPS solution.

3.2 Effects of Various Concentrations and EPS Fractions on Root Growth

The results of different bulk EPS concentration and various fractions of EPS on root growth are shown in Figure 9 and Figure 10, respectively. Bulk EPS solution with a concentration of 50 mg/L is shown to be the ideal concentration for root enhancement. The root weight improved by 39.9% for plants treated with the bulk EPS at 50 mg/L. The weight of root system was not improved as the concentration of bulk EPS solution increases, as shown in Figure 8. Root weight was found to be reduced when using 25 mg/L and 200 mg/L bulk ES solution, which suggests low and high bulk EPS concentration does not enhance root growth.

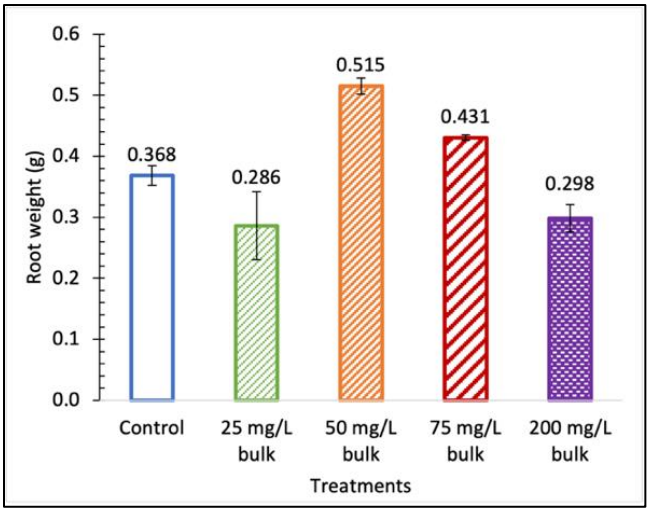

Figure 9. Effects of EPS concentrations on root weight analysis.

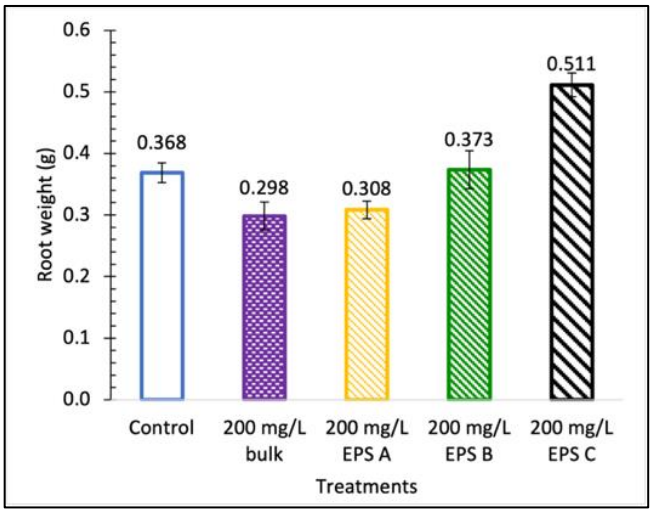

Figure 10. Effects of EPS fractions on root weight analysis.

EPS C solution at 200 mg/L had the highest root weight when compared to other types of EPS at the same concentration (Figure 10). The weight of the root system treated with 50 mg/L bulk EPS is close to the one treated with 200 mg/L EPS C, which suggests that a high concentration is needed for the EPS C to reach a similar result of the bulk EPS (Figures 9 and 10). Seeds germinated with the 200 mg/L bulk and EPS A have lower root weight than the control. A slight increase in root weight was found in the EPS B treatment. This result suggests that 200 mg/L is not the optimal concentration for root enhancement in bulk EPS, EPS A, and EPS B. Further work is in progress to assess the optimal concentration for the different EPS fractions.

3.2 Potted Plant Experiment Results

The weight of 12 rose of sharon plants per group was recorded for plant growth analysis after being treated with 50 mg/L bulk EPS or tap water. Plants watered with EPS solution had greater plant weight than plants watered with tap water (Figure 11). The plant weight results indicate that 50 mg/L bulk EPS solution improve plant growth.

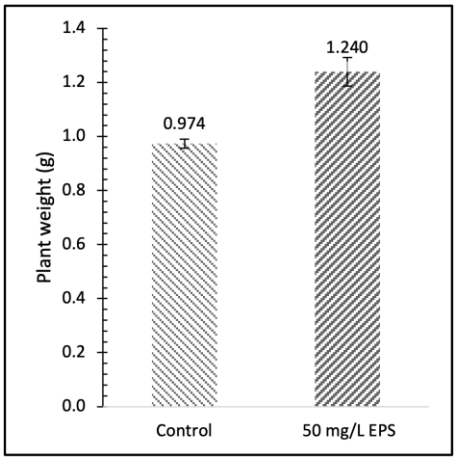

Figure 11. Rose of sharon plants weight.

3.3 Metal Contents Analysis in Plants

XRF Analysis Results on Rose of Sharon Roots

A metal content analysis in rose of sharon roots was conducted using XRF. Roots treated with 50 mg/L bulk EPS solution contained more potassium (K), sulfur (S), and calcium (Ca) than those in the control group (Figure 12). In root growth, K, S, and Ca are essential macronutrients for plant growth and root development. K is a necessary element in root cell expansion [13]. Photosynthesis, respiration, and the production of cell membrane structures require S in plants [14]. Ca is one of the most important elements for cell elongation in both shoots and roots [15]. Lower contents of tungsten (W) and chromium (Cr) were found in EPS experimental group than in the control group. These elements are toxic and non-essential metals for plants, inhibiting plant growth, root elongation and biomass

production [16, 17]. Moreover, high Ca and low W contents were detected in roots treated with EPS solution while low Ca and high W contents were found in the control group. This observation suggests that the EPS biopolymer enhances Ca uptake and prevents W uptake in roots which improves root elongation and development. The presence of tungsten could come from the potting soil used for plant growth. Additional precise characterization method is needed to confirm the tungsten content in the root, as it is relatively small compared to other metal contents.

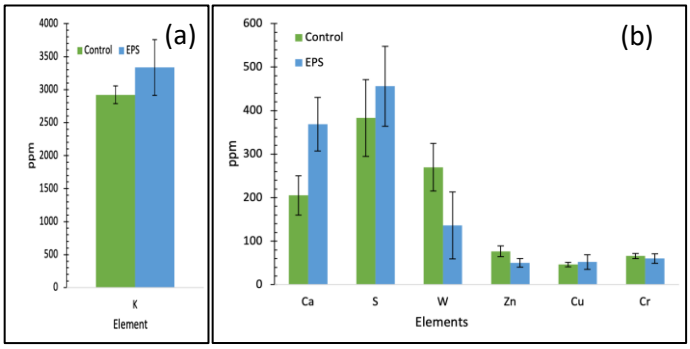

Figure 12. XRF results of rose of sharon roots, (a) metal contents more than 600 ppm, (b) metal contents less than 600 ppm.

XRF Analysis Results on Green Bush Bean Leaves

The XRF results of green bush bean leaves indicate that plants watered with EPS solution consist of higher amount of K, S, and P than plants watered with tap water (Figure 13). As mentioned previously, K and S are essential elements for enhanced plant growth. In addition, phosphorus is one of the commonly used components in fertilizers since it has unique functions in plant metabolism, structure, and reproduction that no other elements can achieve [18]. Furthermore, fruits were produced from green bush bean plants that were watered with EPS solution after 8 weeks of germination while no fruit was produced in the control group (Figure 14). On the other hand, smaller leaves were observed in the EPS watered plants than in the control plants. This observation suggests that EPS promotes flowers and fruits production over leaf growth. XRF analysis of the bean fruits is shown in Figure 15, which indicates that their fruits contain a large amount of K, Ca, P, and silicon (Si). Studies have researched that Si has positive effects on improving crops productivity for sustainable production [19]. Both the XRF results on bean leaves and fruits suggest that the EPS biopolymer accelerates plant growth and fruit production rate by improving essential elements uptake in plants.

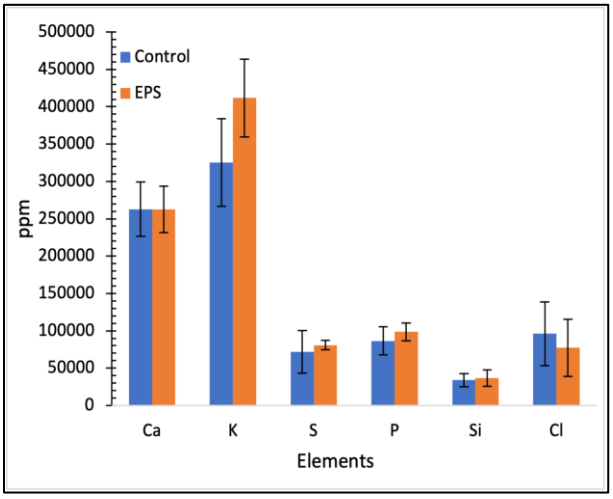

Figure 13. XRF results of green bush bean leaves.

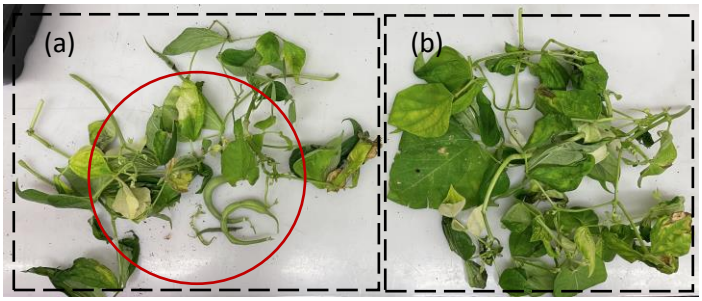

Figure 14. Green bush bean plants watered with a) 50 mg/L bulk EPS solution b) tap water. Note: only EPS watered plants have fruits as shown in the circle.

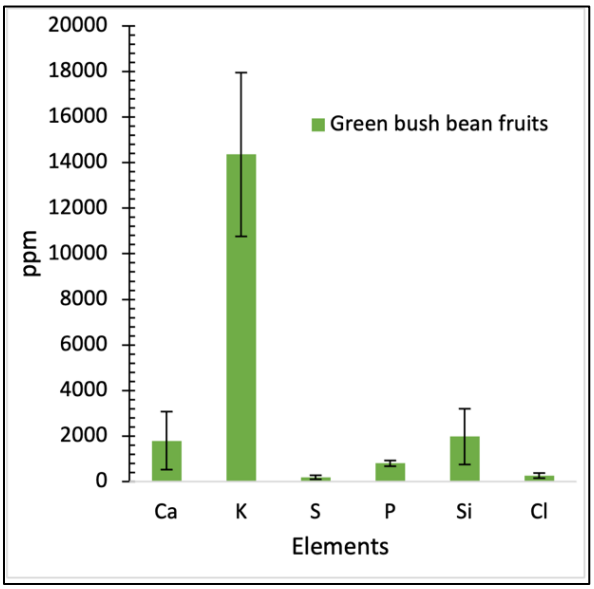

Figure 15. XRF result of green bush bean fruits watered with 50 mg/L bulk EPS solution.

3.4 Characterization Results of EPS Powders

XRF Analysis on EPS Powders

Based on the XRF analysis of the EPS powders in Figure 16, all four fractions of EPS powders contain a large amount of P which is an essential element for plant and root growth. The bulk EPS contains the highest amount of K and Cl among the fractions of EPS. The EPS A has the highest amount of Ca, Si, and iron (Fe). Both the EPS B and EPS C mainly consist of P and K (Figure 15).

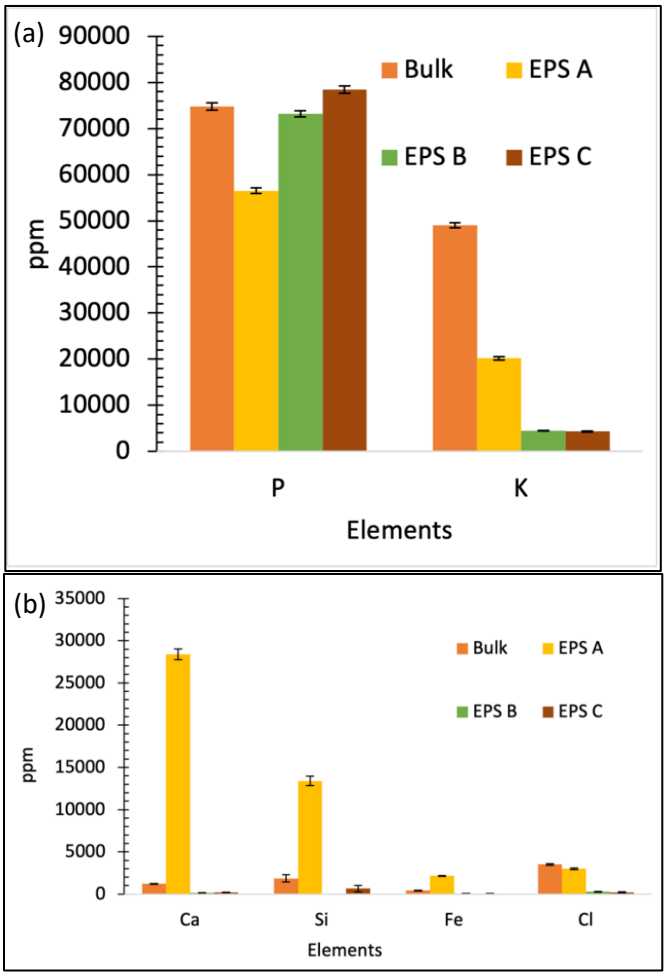

Figure 16. XRF results of EPS powders showing (a) metal contents of P and K, (b) metal contents of Ca, Si, Fe, and Cl.

FTIR Analysis of EPS Powders

Based on the FTIR analysis results of the EPS powders in Figure 17, different bonds and peaks were observed in the four EPS samples. FTIR results also show that the chemical composition is very similar for the three EPS fractions (EPS A, B, C), consisting mostly of carboxylic and hydroxyl groups. Only in the EPS A, both a large amine peak (3291 cm^{-1}) and carbonyl peak (1030 cm^{-1}) were observed, as shown in Figure 17b, which indicates the molecular structure of the EPS A is different from EPS B and EPS C. This fraction is most easily precipitated in ethanol indicating that it has the largest degree of immiscibility in ethanol. This may be from since it has the highest molecular weight, or the slight difference in chemistry due to the amine molecules. The FTIR of the EPS B and EPS C are nearly identical indicating that molecular weight is the primary driver in the precipitation and solubility in ethanol. In addition, bulk EPS mainly consists of EPS B and EPS C.

4. Discussion

From the root analysis results in the germination with various concentration and type of EPS solutions, 50 mg/L bulk EPS was found to be the most effective concentration for green bush bean root enhancement. Based on Figure 9, a negative effect on root growth was detected as the bulk EPS concentration increased to 200 mg/L. One reasonable explanation is that at high concentration, the K content is over the optimal K requirement for root enhancement and that leads to root growth inhibition. It has been reported that root fresh weight of melons decreased when the K levels of nutrient solution was higher than 118 mg/L [20].

EPS C has the most significant root enhancement results in the study of various fractions of EPS at 200 mg/L on root growth. A high concentration is needed for the EPS C to reach a similar root enhancement result in the 50 mg/L bulk EPS. Based on the XRF analysis in Figure 16, the EPS C mainly contains P and K, but the K content is much less compared to the bulk EPS and EPS A. One of the possible explanations for this is that the low K content in EPS C is not sufficient for root enhancement at a low concentration.

The EPS A is expected to be the most effective EPS for root growth since it consists of a larger amount of P, K, and Ca which are essential and beneficial elements for root development and elongation. However, EPS A did not produce greater root growth or enhancement at 200 mg/L. One possible explanation for this is the amount of Ca may exceed the optimal amount for proper root growth. It has been reported that the optimum Ca requirement is 140 mg/L for coffee root growth [21]. Further studies on the optimal concentration and nutrient contents of other three EPS fractions (EPS A, B, C) are required for maximum root growth.

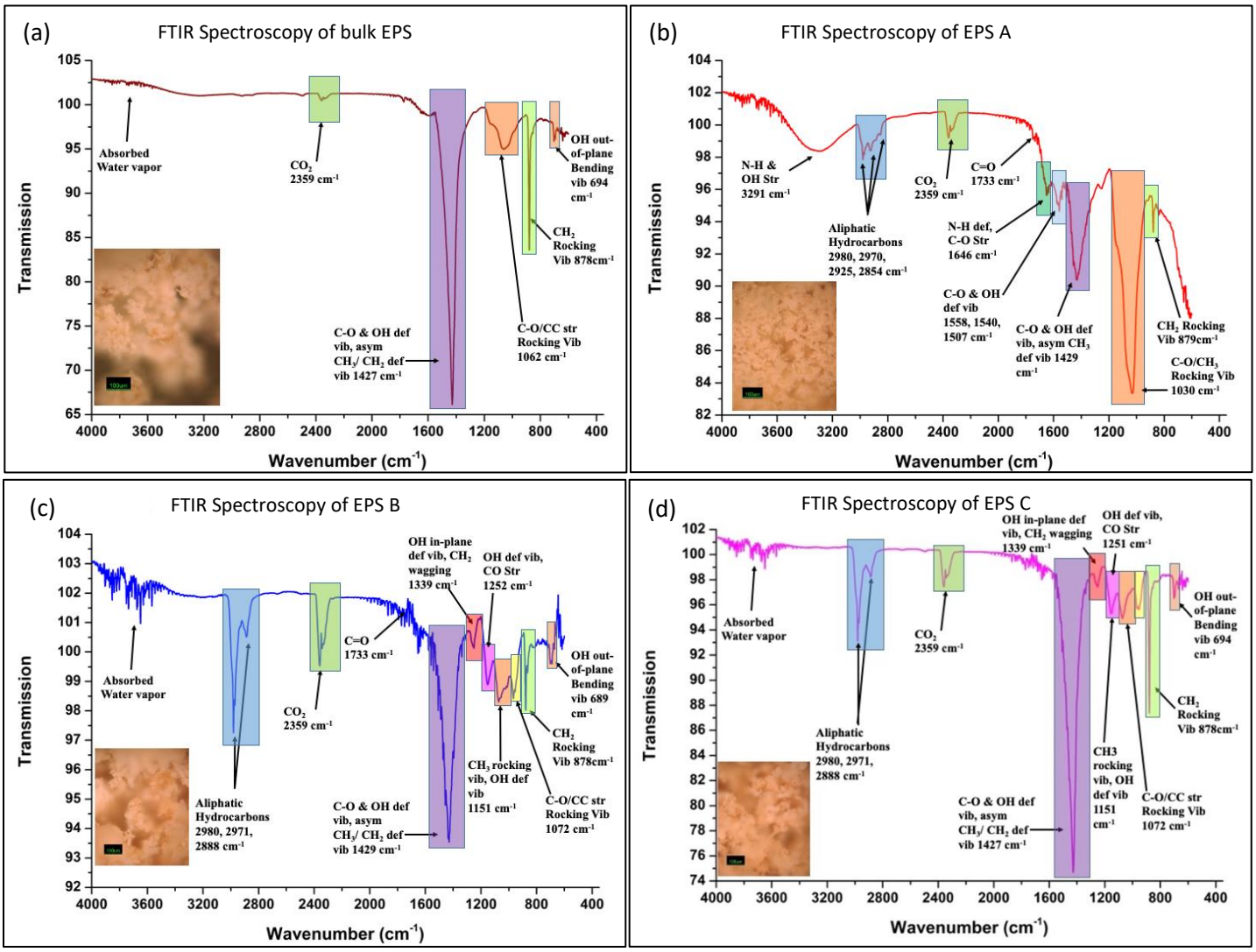

Figure 17. FTIR results and analysis of EPS powders (a) bulk EPS, (b) EPS A, (c) EPS B, (d) EPS C.

FTIR analysis was performed, and several significant peaks were found. In EPS A, there are two significant amine group peaks at 3291 cm^{-1} and 1646 cm^{-1}, and a carbonyl group at 1733 cm^{-1}, however, it has a smaller carboxylic acid group around 1500-1550 cm^{-1}. In EPS B, the amine and carbonyl group are smaller, while the carboxylic acid group is bigger. In EPS C, the amine group and carbonyl group are gone, while the carboxylic acid group is significant. According to the spectra of the four EPS samples, EPS bulk is most likely to consist primarily of EPS B and EPS C, which is characterized by the carboxylic acid group. It also contains a small amount of EPS A, which is characterized by the small amine peak.

Green bean plants watered with 50 mg/L bulk EPS produced fruits after 8 weeks of germination. XRF results on the fruit shows that Si is the 3rd highest element in the fruits, and Si is an essential micronutrient for fruit production. In addition, some amount of Si was detected in the bulk EPS by XRF analysis, and the result suggests 50 mg/L bulk EPS accelerates fruit production rate by improving Si uptake in plants.

5. Conclusions

EPS fractions were precipitated with different ratio of ethanol to biopolymer. FTIR results on EPS powders indicate that the bulk EPS is different from the three EPS fractions (EPS A, B, C), and the three fractions share similar molecular structure. The EPS A sample indicated prominent carbonyl and amine peaks, which were absent in the other fractions.

XRF analysis indicates that all four EPS samples contain a large amount of P, and the bulk EPS has the highest amount of K. XRF analysis on plants shows bulk

EPS treated plants have more K contents than plants without EPS treatment, and this result is consistent with the high K level in bulk EPS.

Bulk EPS added to regional water at a concentration of 50 mg/L was shown to enhance growth in both green bush bean and rose of sharon plants. The plant weight was increased by 27.3% for the root weight of rose of sharon. For green bush beans germinated with EPS solution, the number of tertiary roots was increased by 200%. Moreover, bean plants only watered with EPS produced fruit after 8 weeks of germination, which suggests EPS accelerates flower and fruit production over leaf growth. overall, the EPS produced by *R. tropici* enhances root growth and root branching for developing a rich root system and suggests a strong possibility for soil erosion resistance.

Watering the bean plants with different fractionations at a concentration of 200 mg/L produced the highest root weight for EPS C. Comparing concentrations in the watering mixture for the bulk showed maximum root system at a concentration of 50 mg/ml. Hence addition of EPS enhanced all aspects of plant growth and can be a viable alternative to fertilizers. Further study for discovering the ideal concentration and nutrient content of the three EPS fractions (EPS A, B, C) for optimal root growth is in progress.

6. Acknowledgements

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