Research Article
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The influence of *Rhizobium tropici* produced EPM biopolymer on green bush bean root and plant growth

Abstract

A *Rhizobium tropici (R. tropici)* derived biopolymer has been reported as an effective, biodegradable, additive to reduce erosion. In addition to directly modifying the mechanical properties of the soil, it was postulated that indirectly it enhanced vegetation, which in turn stabilized the soil through enhanced root infrastructure. We therefore chose to study its impact directly on Green Bush Bean plants, where its effect could be measured in the early stages of plant development, starting from germination of the seeds through the initial growth of leaves and shoots. EPM derived from *R. tropici* bacteria ATCC (strain) grown in two laboratories were tested with similar results, indicating a high degree of reproducibility. Watering Bush Bean seeds with EPM concentrations as low as 50 and 100 mg/L produced a small increase of the germination rate, from 87% to 93% for seeds grown for ten days in a moist environment. The seeds were then transplanted into potting soil and allowed to grow for another three weeks, during which they were watered daily with tap water or the EPM solutions. Continued watering with EPM after transplantation showed enhancement in both root and stem/leaf mass three weeks after transplantation into potting soil by 45% for the two EPM biopolymers. Root density was also higher by 29% to 71% for EPM1.The mass of the leaf and shoots also showed a significant enhancement over the control, but in this case favoring EPM2, consistent with plants preferring either root or leaf production.

Keywords: EPM biopolymer, *Rhizobium tropici*, soil erosion, root growth, plant growth, fertilizer

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Introduction

Soil erosion is often seen in the loss of top layer soil leading to the exposure of layers beneath the surface by Evans. The detriments of soil erosion involve decreases in crop yield as well as runoff to other locations.[1] The effects of climate change are currently observed around the world, with more frequent and severe weather events, it has increased soil erosion rates.[2] To address this issue, plant development both aboveground and belowground is crucial in holding soil in place. Numerical studies have shown that the percentage of vegetation cover in an area is able to slow the soil erosion rate.[3] Plant roots also contribute to the soil's system of continuous pores and enhance its infiltration capacity, decreasing soil erosion rate.[4] Fertilizers are the traditional method for enhancing vegetative growth, but chemical fertilizers are also known to pollute waterways, increase nitrogen compounds, and disturb the natural biodome. Hence much effort is currently being invested in finding alternative means to enhance plant growth and mitigate soil erosion through application of environmentally sustainable materials.

Extensive field studies have demonstrated that biopolymers produced from *R. tropici*-derived extracellular polymeric substances (EPS) decrease the soil erosion by enhancing slope stability and preventing run off.[5] Analysis of the soil samples, six months after application did not show evidence of the biopolymer, and hence the beneficial aspects were attributed to early impact of the biopolymer on enhancing germination and root development which all contribute to soil retention.[5] The studies reported by the Engineer Research and Development Center (ERDC) group focused on the mechanical properties of the soil correlated to different treatment modalities of berms with the biopolymer and its impact on the vegetation was only inferred from the outcomes. We therefore designed the current studies to quantitatively investigate the impact of the biopolymer on the early stages of plant growth by measuring the effect on germination, and mass of the root and shoots.

The plants we chose to study were green bush beans, since they have a relatively short time to germination of 7-12 days ("Non-GMO and Heirloom Contender Bush Bean Seeds").[7] The biopolymers we chose were two ethanol precipitable materials (EPM) biopolymers derived from *R. topici* which were produced under the same cultivation environment and procedure, but from two different sources of the same bacteria.

Materials & methods

Plants materials

Green bush bean species were used in the germination and plant growth experiments. And the seeds were purchased from the Sow Right Seeds brand.

R. Tropici Strains

The lots of *Rhizobium tropici* ATCC 49672 were compared. One was received from the ATCC (American Tissue Culture Collection) ATCC 49672, LOT 58473611, and one was received as "*Rhizobium tropici* CIAT 899 (UMR 1899)" from Dr. Michael Sadowsky, Director Bio Technology Institute, Distinguished McKnight Professor, University of Minnesota. EPM produced from the *Rhizobium tropici* ATCC 49672, LOT 58473611 was referred as EPM 1 and EPM produced from the *Rhizobium tropic* CIAT 899 (UMR 1899) was referred as EPM 2. All EPM used in this study were produced in Stony Brook School of Dental Medicine.

Ethanol precipitable material (EPM) preparation

The EPM was prepared according to the procedure described by Staudt et al.,[6] with ethanol removed using a speed-vac rather than heating at 50 °C to avoid caramelization. The detailed procedures on EPM preparation are described as the following steps. A single clone from a peptone yeast extract agar plate was inoculated into 10mL peptone yeast medium (0.5% peptone of casein, 0.3% yeast extract, and 10 mM $CaCl_2$) and cultured for 24 hours at 30°C, stirred at 300 rpm. Cells were then transferred into 250 mL of liquid minimal medium (LMM) containing 2g/L NH_4Cl, $MgSO_4$-$7H_2O$, 0.15 g/L $CaCl_2$-$2H_2O$, and 10 mg/L $FeCl_3$ in 50 mM potassium phosphate buffer (pH 6.9) and 55 mM glucose. Cells were grown in a heated stirrer for another 48 hours (30°C, 300 rpm). Cells were then removed by centrifugation at 10,000 g for 15 minutes, and the supernatant was transferred into a clean vessel. The EPM was then prepared by adding three volumes of ethanol; the solution was chilled overnight at 4°C. The floating EPM was collected into a sterilized tube, which was then centrifuged at 3,000 rmp for 15 minutes. The pellets were washed with 70% cold ethanol and dried in a tube for speed-vac (avoids caramelization).

EPM solution preparation

EPM solutions of different concentrations were prepared by dissolving the desired amount of EPM powders into 1 liter of tap water. Tap water was used as a solvent in the EPM solutions preparation rather than DI water, since tap water was used as the control treatment throughout the study. To prepare the treatment solutions, the biopolymer in powdered form was first measured using a weight boat and a scale accurate to the hundredths of a milligram, as the concentration required a high precision. A one-liter flask was filled with some tap water first, then the respective powder was added to the flask, and finally the flask was filled to the one-liter mark. The solutions were mixed sufficiently using a magnetic stirrer and sealed with parafilm for later use. EPM solutions of two different concentrations prepared from the two different EPM biopolymers were used to study the effects of EPM concentrations and strains on seed germination and plant growth: 50 mg/L EPM 1, 100 mg/L EPM 1, 50 mg/L EPM 2, and 100 mg/L EPM 2. The pH of the tap water was 7.5, and all EPM solutions has a pH value of 7.5±0.1.

Germination and plants growth conditions

All seed germination and plant growth experiments were carried out under growing lights in greenhouses, as shown in Figure 1. The wavelengths of growing light are between 430 to 470 nm, blue light and between 600 to 800 nm, red light. The temperature inside the greenhouse was maintained at room temperature, 25±3°C.

Figure 1 Greenhouses with growing lights.

Germination and plant growth experiment

Green bush beans are divided into five groups: control (tap water) and four EPM treatments. For the germination experiment, three petri dishes were used for each treatment, with five seeds on each petri dish, watered with 50 mL of tap water or treatment solution daily. The four treatment solutions were: 1) T1: 50 mg/L EPM 1, 2) T2: 100 mg/L EPM 1, 3) T3: 50 mg/L EPM 2, 4) T4: 100 mg/L EPM 2. After 10 days of germination, the green bush bean seedlings were transplanted into soil. In addition, the germination rate was calculated based on equation 1.

$$Germination\,rate = \frac{\#of\ seeds\ germination}{total\ \#of\ seeds\ sown} \times 100\%$$

The five groups of green bush bean plants were watered with the desired treatment solution for three weeks. The pH and moisture level of soils in each group were detected and monitored by a soil pH / moisture detector (Sonkir 3-in-1 Soil Moisture Light pH Tester), as shown in Figure 2. The soil pH level was maintained at 7.5±0.5, and the soil moisture level was maintained between the level of "moist" as shown on the detector display.

Figure 2 Sonkir 3-in-1 soil moisture light pH detector.

Analysis of the effects of each treatment by separately measuring the masses and lengths of the roots and stems and leaves of each bean were conducted. Mass measurements were used to investigate whether the EPM biopolymer influenced the growth of the roots versus the stems and leaves differently. As such, the green bush bean plants were removed from soils for roots and plants analysis after three weeks of transplantation. The soil was washed off and the plants were dried completely to ensure that the mass was solely of the plant.

The masses were then measured with a scale accordingly. Upon initial analysis, mass differences between treatments were observed, and a further investigation took place on whether the mass differences were due to density or height. Images of each individual plant and used ImageJ were taken to measure the length of each one.

Results & discussion

Germination rates

In Figure 3a we plot the germination rate from seeds placed on a moist towel in 120 mm petri dishes, and placed in a greenhouse, under ambient conditions. The seeds were watered daily with 40 mL of either tap water or tap water with either 50 mg/L or 100 mg/L of EPM1 or EPM 2. The samples are shown in Figure 3b. From the figure we find that at ten days significant root structure was already

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apparent in most seeds. The germination rate, which was defined was the fraction of seeds showing shoots vs the total for the seeds placed in the respective dishes, is plotted in figure 3a, where we can see that despite large difference in root length between samples, no significant differences in the number germinated plants, could be observed.

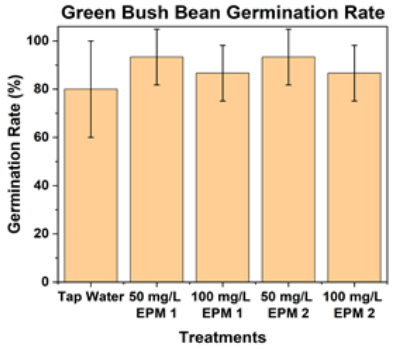

Figure 3a Germination rate of green bush bean.

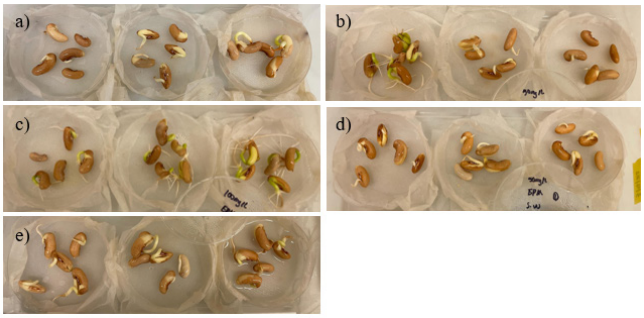

Figure 3b Germination of green bush beans in petri dishes (day 5), a) tap water, b) 50 mg/L EPM 1, c) 100 mg/L EPM 1, d) 50 mg/L EPM 2, and e) 100 mg/L EPM 2.

Root growth

Each germinated seed was then placed in a pod of soil, 2 cm times 2 cm wide and 7 cm high. The seeds were then watered daily with tap water or with the EPM solutions. The moisture content of the soil was monitored as described previously. The plants are shown after three weeks in Figure 4, where we can see that all the transplanted seeds developed into plants with shoots and leaves. The plants were then removed from the soil, rinsed repeatedly with tap water to remove residue, and the roots were cut from the stem. This is illustrated in Figure 5, which shows a typical plant after rinsing. The red line indicates the position where the roots were cut from the shoot. The roots corresponding to the different watering conditions are then shown in Figure 6a. The mass of the root sections was then measured by weighing each plant separately and averaging the weight of the roots within each of the five groups. The results are plotted in Figure 6b. From the figure we can see that the root mass corresponding to EPM2, was 14% larger than the control, and no significant difference between the 50 and 100mg/L concentrations as detected. The increase relative to the control was larger for the EPM1, where 29% was observed for the 50 mg/L and 71% observed when the concentration was increased to 100mg/L. Comparison of the root symptoms shown in Figure 5b indicates that the root systems watered with either EPM1 or EPM2 are denser and have more branches than those water with the control. This is consistent with a previous study which found a direct correlation between root weight and branching density.[8] Hence here as well we find that treatment with water containing EPM enhances the root structure of the plants.

Figure 4 Comparison of green bush bean plants a) tap water, b) 50 mg/L EPM 1, c) 100 mg/L EPM 1, d) 50 mg/L EPM 2, and e) 100 mg/L EPM 2.

Figure 5 Illustration of green bush bean plant after rinsing.

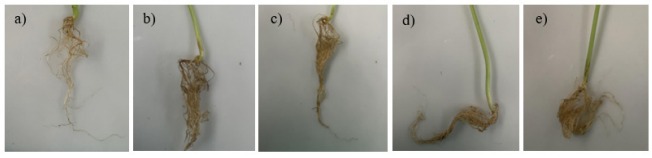

Figure 6a Comparison of green bush bean root system a) tap water, b) 50 mg/L EPM 1, c) 100 mg/L EPM 1, d) 50 mg/L EPM 2, and e) 100 mg/L EPM 2.

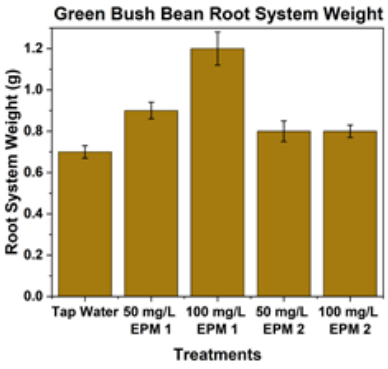

Figure 6b Root system weight of green bush bean plant.

Leaf and stem analysis

Stem heights of green bush bean plants are determined by measuring the length of the green stem from the top of the root to the tip of its longest branch. The lengths are measured using the measurement tool on Image J. Mean and standard deviation of each treatment group are calculated to compare each EPS treatment group with the control group. A bar graph comparing the mean stem height in each EPS treatment group is plotted in Figure 7a. The result shows that there was a 21% to 43% increase in plant height that was treated with EPM solution.

The mass of the green bush bean stem and leaves was also measured for every plant. The data is plotted in Figure 7b where we can see

that the plants treated with either of the EPM solutions have biomass that is higher than the control by 45% and 58% for EPM1 and EPM2 respectively. The slightly lower leaf and stem biomass observed for EPM1 is compensated by the larger root biomass observed for this formulation.

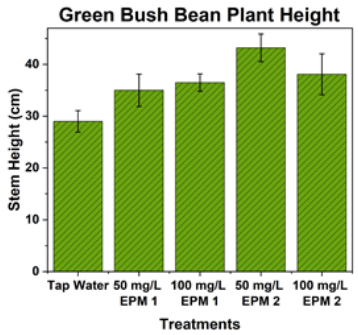

Figure 7a Plant height of green bush bean plant.

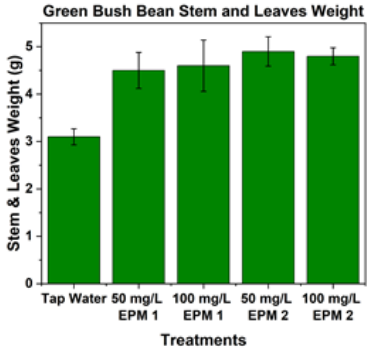

Figure 7b Stem and leaves weight of green bush.

Conclusions

The impact of low concentrations of *R. tropici* EPM on germination and growth of bush bean plants was measured. EPM derived from *R. tropici* bacteria grown in two laboratories were tested with similar results. The data show that concentrations as low as 50 and 100 mg/L significantly enhanced germination times after ten days for seeds grown in a moist environment. Continued watering with EPM after transplantation showed enhancement in both root and stem/leaf mass three weeks after transplantation into potting soil. Root density was significantly higher than the control for both EPM1 and EPM2, with EPM1 resulting in higher mass. Similarly measuring the mass of the leaf and shoots also showed a significant enhancement over the control, but in this case favoring EPM2, consistent with plants preferring either root or leaf production. These results suggest that the EPM biopolymer stimulates bush bean plants to develop a denser root structure and a larger leaf, and shoot system, which enhances plant survival. Taken together, these results may explain the reported increase in resistance to soil erosion when biopolymer was used to irrigate different berm slope sites. [9–11]

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Conflicts of interest

Authors declare that there is no conflict of interest.

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