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# Evaluation of *Rhizobium tropici*-Derived Extracellular Polymeric Substances on Selected Soil Properties, Seed Germination, and Growth of Black-Eyed Peas (*Vigna unguiculata*)

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## Abstract

*Rhizobium tropici*-derived extracellular polymeric substances (EPS) have been used in soils to enhance soil structures and mitigate soil erosions. However, information on their use to improve soil health and fertility indicators, and plant growth is limited. In a greenhouse study, we investigated their effects on some soil health, soil fertility indices, and the growth of black-eyed peas (*Vigna unguiculate*). Results showed that soils incubated with EPS significantly increased basal soil respiration, soil microbial biomass, permanganate oxidizable carbon (POC), and potentially mineralizable nitrogen (PMN). The EPS shifted microbial populations from bacteria to fungi and Gram (−ve) to Gram (+ve) bacteria. However, it had little or no effects on soil pH, soil organic matter (SOM), and cation exchange capacity (CEC). The EPS decreased soil moisture loss, increased soil aggregate stability, but delayed blacked-eyed peas germinations in the soils. At 0.1% (w/w) concentrations in soils, there was increase in plant root nodulations and vegetative growth. This study was carried out within 40 days of incubating soils with EPS or growing the black-eyed peas in a greenhouse study. The plant growth parameters were taken before flowering and fruiting. Further studies of the effects of incubating soils with the extracellular polymeric substances on plant growth. Soil microbial biomass, microbial diversities, and other soil fertility indices are deemed necessary.

## Keywords

*Rhizobium tropici* Extracellular Polymeric Substances, Soil Respiration, Soil

Microbial Biomass, Black-Eyed Peas

## 1. Introduction

*Rhizobium tropici* is a legume-symbiont soil bacterium known for its potential to produce extracellular polymeric substances (EPS) and fundamental biofilm constituents [1]. Its symbiotic relationship with legumes in biological nitrogen fixation contributes to plant nitrogen nutrition and carbohydrate acquisitions from the plant [2] [3]. The EPS are composed mainly of polysaccharides, proteins, lipids, humic substances, uronic acids, minerals, and DNA; but its chemical compositions may vary within growth media [4] [5]. *Rhizobium tropici* is known to produce copious amounts of EPS and due to its high multiplication rates, various techniques have been employed for its culture in various media [6] [7]. EPS have been reported to have the potential to aggregate soil particles [8], strengthen soil berms, and mitigate soil erosion [9]. However, despite numerous studies [10] [11] [12], their evaluations and effects on soil fertility indicators and plant growth need further studies. This study was designed to evaluate the use and effects of *Rhizobium tropici*-derived extracellular polymeric substances on soil health and fertility indicators and growth of black-eyed peas (*Vigna unguiculata*), an edible legume, considered a decent source of carbohydrates, proteins, beta carotene (precursor of vitamin A), and fiber [13] [14].

## 2. Materials and Methods

### 2.1. Soil Sampling and Analysis

The soil used in this study is the Alabama Decatur silt loam soil classified as fine, kaolinitic, thematic, Rhodic Paleudult [15] which was sampled and passed through a 2-mm sieve and stored at 4˚C before analysis. The climate in the region is subtropical, with a mean annual rainfall of 1355 mm and a mean annual temperature of 17˚C [16]. The soil has a particle size distribution of 600 $g \cdot kg^{-1}$ sand, 300 $g \cdot kg^{-1}$ silt, and 100 $g \cdot kg^{-1}$ clay, and was not under crop production nor has been fertilized for years prior to sampling. The soil properties determined included soil aggregate stability, exchangeable cations, wet soil aggregate stability [17], soil organic matter [18], soil organic carbon [19], soil pH measured in a 1:2.5 soil:water and 0.01 *M* $CaCl_2$ suspension, permanganate oxidizable active carbon (POC) [20], and potentially mineralizable nitrogen (PMN) [21]. Cation exchange capacity (CEC) was determined after exchange with ammonium acetate ($NH_4OAc$) and the $Ca^{2+}$, $Mg^{2+}$, $K^+$, $Na^+$, $H^+$, and $Al^{3+}$, determined in the extracts using inductively coupled plasma optical emission spectrometry (ICP-OES). Phospholipid fatty acid (PLFA) analysis technique was used in estimating total microbial biomass and changes in the soil microbiota community compositions. The EPS used, (produced by *Rhizobium tropici* ATCC® 49672™) was obtained

from the United States Army Engineer Research and Development Center, Environmental Laboratory, Vicksburg, Mississippi, USA (Patent No US7.824, 569 B2). The EPS was weighed (taking into consideration the dry mass) and dissolved in deionized water before mixing with a weighed amount of soil. The amount of water used to dissolve the EPS depended on the desired moisture content of the biopolymer-enriched soils.

## 2.2. Soil Respiration

One hundred gram of moist soil (29% moisture content) on an oven-dried weight basis was incubated with EPS (0.5% w/w) and 2 mm corn leaves (0.15% w/w) and incubated in Erlenmeyer bottles at 25˚C for 5 weeks. Soil respiration was determined by measuring the $CO_2$ released and absorbed in NaOH [22]. At the end of each week, the NaOH in each vial was quantitatively transferred into individual 250 mL Erlenmeyer flasks using several rinses. Thirty mL 2*N* $BaCl_2$ was then added to NaOH to react and precipitate carbonate ions as $BaCO_3$. Three drops of phenolphthalein indicator were added to the contents of the flask and immediately titrated while swirling, with 0.1 *N* HCl to a white endpoint. The $CO_2$ released in mg $CO_2$ per 100 g soil was calculated as follows: mg $CO_2$ = [meq. base − meq. acid] × 22.

## 2.3. Seed Germination and Plant Growth in Soils

Soil was incubated with 0.5% EPS (w/w) and 5 black-eyed peas seeds planted in pots containing 1800 g soil (oven-dry weight) maintained at 29% moisture in the greenhouse. The 0.5% (w/w) rate for EPS was chosen based on previous studies on Atterberg limits geotechnical soil properties (**Table 1**). The Liquid Limit (LL), also known as the upper plastic limit, is the water content at which the soil changes from the liquid state to a plastic state. It is the minimum moisture content at which soil flows upon application of minimal shear force. The Plastic Limit (PL), also known as the lower plastic limit, is the water content at which a soil changes from the plastic state to a semisolid state. The Plasticity Index (PI) was calculated as the Plastic Limit subtracted from the Liquid Limit (LL). PI = LL − PL.

There were 5 pots per treatment, giving a total of 25 seeds that were allowed to

**Table 1.** *R. tropici*-derived extracellular polymeric substances (EPS) on Atterberg Limits.

| Soil Amendment | Liquid Limit (LL) % | Plastic Limit (PL) % | Plasticity Index (PI) |
|---|---|---|---|
| 0% EPS | 41.14 ± 0.92$^{c}$ | 20.75 ± 0.76$^{a}$ | 20.39$^{c}$ |
| 0.1% EPS | 42.1 ± 0.82$^{b}$ | 20.63 ± 0.25$^{a}$ | 21.47$^{b}$ |
| 0.5% EPS | 47.3 ± 0.36$^{a}$ | 20.46 ± 0.21$^{a}$ | 26.84$^{a}$ |

*Mean values with similar letter indicate not significantly different based on Tukey's Studentized Range (HSD) test at $\alpha = 0.05$.

germinate. Ten days after planting, the most viable (healthiest looking) BEP seedling in each pot was allowed to continue growing while the rest were removed, leaving 5 plants per treatment. The number of seeds that germinated during the experiment were noted and recorded. The moisture content was maintained by weighing the potted soil every two days, and the loss in weight was compensated by adding the equivalent amount of water. The pots were rotated every two days to minimize the effects of the phototropic response of the plants. The experiment was repeated by incubating soils with 0.02 and 0.1% EPS (w/w) and comparing the plant growth parameters with non-incubated soils.

Plant performance was assessed after every two days by measuring the number of leaves per plant, leaf area (LA), and stem height. Thirty-one (31) days after planting, the plants were carefully removed and the root lengths, number of root nodules per plant, shoot, and fresh root weights were recorded for each treatment. Plant dry weight was obtained by placing the plant materials in an oven at 105˚C for 1hr and then at 40˚C for 24 hrs. Leaf area (LA) measured in $cm^2$ was estimated using a non-destructive linear model: LA = 0.463 + 0.676WL equation [23]. Leaf length (L) was measured from the leaf tip to the point at which the lamina was attached to the petiole, while maximum leaf width (W) at the widest point perpendicular to the lamina mid-vein was measured to the nearest millimeter.

### 2.4. Soil Microbiota

Soil incubations with EPS and BEP growth on soil microbial biomass and microbial community structure were evaluated using the phospholipid fatty acid (PLFA) analysis techniques [24] [25] [26]. The PLFA analysis included lipid extraction and fractionation, and GC-MIDI analysis using the method suggested by Buyer and Sasser [24]. The PLFA profiles were prepared following the MIDI protocol, analyzed based on the MIDI Sherlock® Microbial Identification System (MIDI, Newark, DE, USA), and separated using gas chromatography (Agilent 6850, Agilent Technologies, USA) in combination with flame ionization detector techniques on capillary column ultra-2, and a split ratio of 30:1. All the results obtained are reported on a dry weight basis.

### 2.5. Data Analysis

The data collected was analyzed using two sample t-tests or ANOVA and General Linear Model (GLM) procedures of the SAS 9.4 for Windows (SAS Institute Inc. Cary, NC, USA). The means were separated using the Tukey's Studentized Range (HSD).

## 3. Results

### 3.1. Soil Respiration and Microbial Biomass

Soil respiration significantly increased in soils incubated with EPS and was significantly enhanced when the EPS was supplemented with corn leaves (CL)

(Figure 1). The soil respiration rates of incubated soils shown in Figure 2 decreased sharply from week 1 to week 2 and then gradually to week 5. Table 2 shows the effects of incubating soils with EPS and corn leaves on soil microbial biomass and microbial compositions. The results showed that EPS increased the

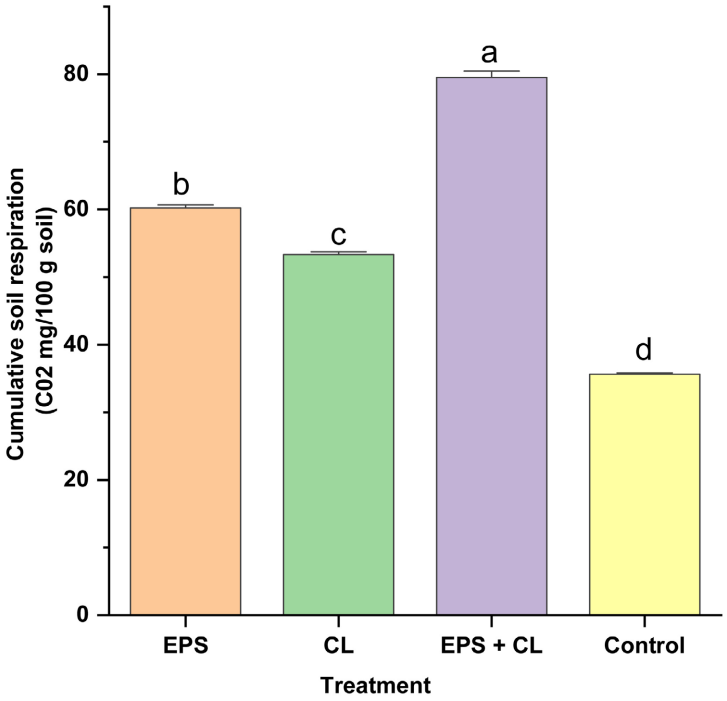



Figure 1. Relationship between soil respiration and EPS, corn leaves (CL), EPS + CL after 5 weeks of incubation. Letters indicate means statistically different based on Tukey's Studentized Range (HSD) comparison with $\alpha = 0.05$.

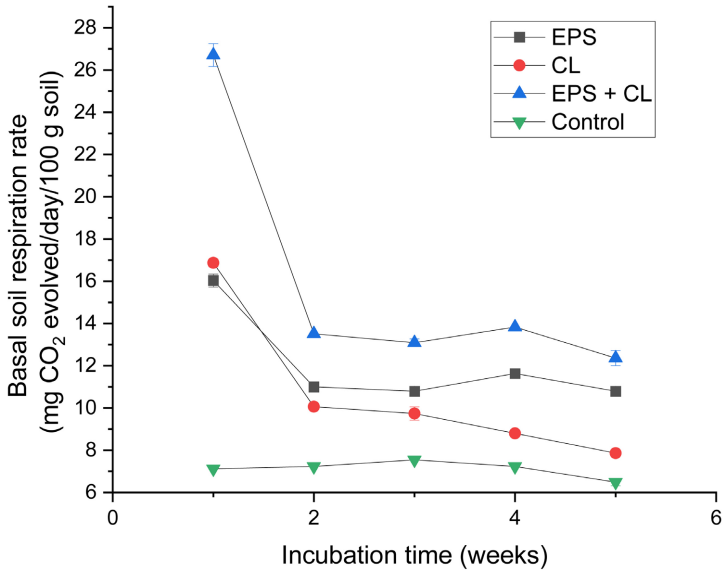



Figure 2. Relationship between soil respiration and soils incubated with EPS, corn leaves, EPS + corn leaves.

**Table 2.** Soil incubation with EPS and corn leaves (CL) on soil microbial biomass and microbial groups (fungi, arbuscular mycorrhizal fungi, AMF, G (−) and G (+), bacteria, eukaryotes, and actinomycetes).

| Soil amendment | Microbial biomass (pmol/g soil) | | | | | | | | Microbial biomass ratios | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total PLFA | Total NLFA | Fungi | AM Fungi | Gr (−) | Gr (+) | Eukar | Actinos | F/B | Gram+/− | Gr-stress | Total NLFA/ PLFA |
| Control | 58,967 | 80,678 | 647 | 1675 | 20,253 | 8945 | 3020 | 9152 | 0.08$^{c}$ | 0.89$^{c}$ | 0.64$^{c}$ | 1.37$^{b}$ |
| EPS | 93,103 | 91,849 | 1368 | 3347 | 32,096 | 19,336 | 1388 | 12,185 | 0.09$^{b}$ | 0.99$^{a}$ | 1.13$^{a}$ | 0.99$^{c}$ |
| % Change | 57.9% | 13.8% | 111.4% | 99.8% | 58.5% | 116.2% | 54.0% | 33.1% | | | | |
| CL | 61,775 | 89,490 | 1534 | 1959 | 24,203 | 11,272 | 612 | 10,916 | 0.1$^{a}$ | 0.92$^{b}$ | 0.72$^{b}$ | 1.45$^{a}$ |
| % Change | 4.8% | 10.9% | 137.1% | 16.9% | 19.5% | 26.0% | 79.7% | 19.3% | | | | |
| EPS + CL | 96,402 | 84,774 | 1944 | 3442 | 33,864 | 21,178 | 1390 | 13,091 | 0.1$^{a}$ | 1.02$^{a}$ | 1.13$^{a}$ | 0.88$^{d}$ |
| % Change | 63.5% | 5.1% | 200.5% | 105.5% | 67.2% | 136.8% | 53.9% | 43.0% | | | | |
| CV | 25.7 | 5.7 | 39.4 | 35.3 | 23.4 | 39.4 | 63.2 | 15.1 | | | | |

*Mean values with similar letter indicate not significantly different based on Tukey's Studentized Range (HSD) test at $\alpha = 0.05$.

total biomass (PLFA) and the major microbial groups, particularly fungi and arbuscular mycorrhizal fungi (AMF) biomass. The increase in microbial biomass was also greatly enhanced by combining the EPS with 2 mm corn leaves (CL). The microbial biomass ratios, EPS tends to shift the microbial community structure from bacteria to fungi and from Gram (−ve) to Gram (+ve)) bacteria after five weeks. The results suggested that EPS significantly increased the ratio of monounsaturated fatty acids to cyclopropane fatty acids (16:1Ω7c + 18:1Ω7c)/(cy17:0 + cy19:0) measured as the Gram (−ve) stress and the higher the value, the lower the environmental stress. The coefficient of variation (CV) values indicated that fungi, AMF, and Gram (+ve) bacteria varied increasingly due to soil perturbations and incubations than the other microbial groups.

### 3.2. Evaluation of *R. tropici*-Derived Extracellular Polymeric Substances on Seed Germination and Plant Growth in Soils

Figure 3 shows the results of incubating soils with EPS on seed germination 5 days after sowing. The number of bean seeds that germinated in incubated and non-incubated soils was monitored daily for 17 days. Results showed that EPS (0.5% w/w) delayed BEP seed germination in the potted soils and a germination rate of 68% and 88% respectively was observed for soils incubated with 0.5% EPS (Table 3). At lower EPS concentrations in soils (0.02% and 0.1% w/w), it was observed that EPS delayed seed germination (Table 3). The growth parameters of the plants grown in EPS-incubated and non-incubated soils are shown in Table 4 and Figure 4. Two-sample t-tests (assuming equal variances), showed that after 31 days of plant growth, the stem height, root length, and shoot biomass, although higher for plants grown in non-incubated soils, the values were not


**Figure 3.** *R. tropici* EPS on seed germination 5 days after sowing at 0.5% (w/w) EPS and 0% EPS.

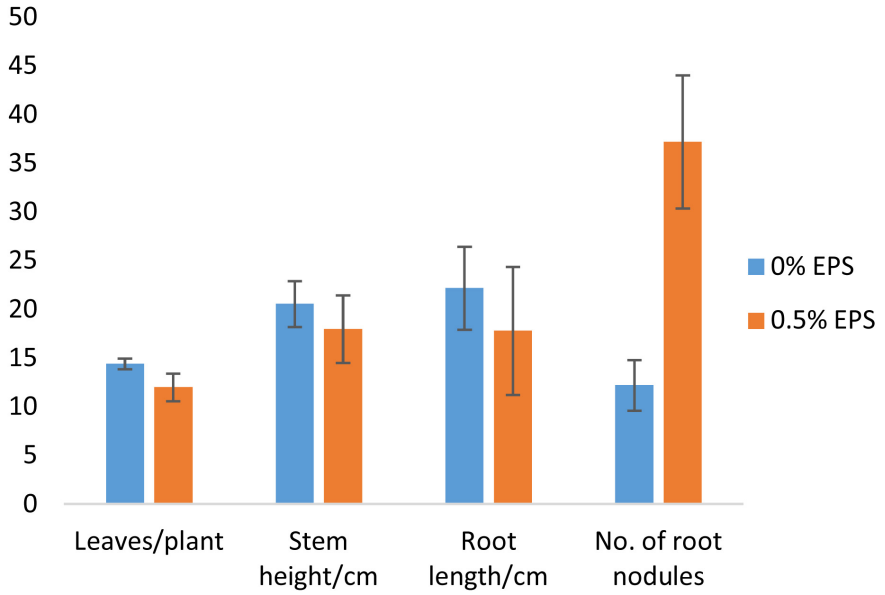



**Figure 4.** Relationship between *R. tropici* EPS at 0 and 0.5% w/w with plant growth.

**Table 3.** Seed germination rates in EPS-incubated soils.

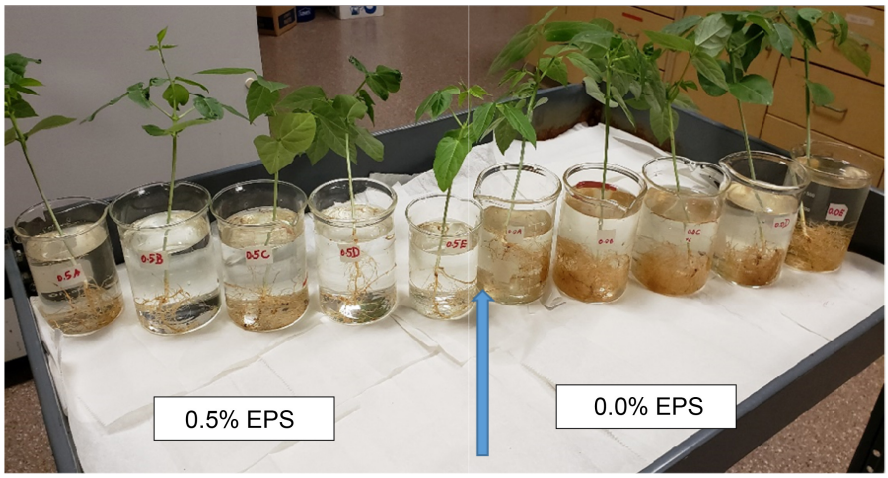
| Percent EPS in soil | % Seed germination in soils | | |
|---|---|---|---|
| | Day 5 | Day 6 | Total after 10 days |
| 0 | 80 | 88 | 88 |
| 0.02 | 36.9 | 58.7 | 80 |
| 0.10 | 18.5 | 58.7 | 72 |
| 0.50 | 18.5 | 48.0 | 68 |

**Table 4.** Plant biomass at 0 and 0.5% EPS incubation.

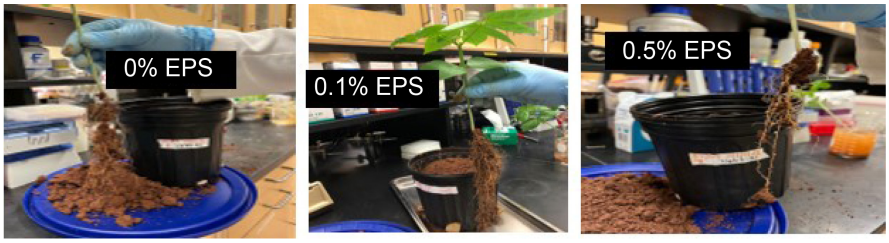
| Soil incubation | Average leaf area per plant/cm$^2$ | Shoot biomass/g dry mass | Root biomass/g dry mass | Root/Shoot dry mass ratio |
|---|---|---|---|---|
| 0% EPS | 175.2 ± 39.54 | 1.098 ± 0.265 | 0.274 ± 0.026 | 0.261 ± 0.061 |
| 0.5% EPS | 118.8 ± 7.69 | 0.804 ± 0.152 | 0.154 ± 0.013 | 0.195 ± 0.022 |
| t statistic (df), *p* value | t(8) = 3.13, p > 0.0139 | t(8) = 2.15, p > 0.0634 | t(8) = 9.23, p < 0.0001 | t(8) = 2.31, p > 0.049 |

significantly different from plants grown in soils incubated with 0.5% EPS. The number of leaves per plant t(8) = 3.54, P = 0.0076, leaf area t(8) = 3.13, P = 0.0139, root biomass t(8)= 2.15, p = 0.0634, and root/shoot dry mass ratio t(8) = 2.31, p = 0.049, were significantly higher for plants grown in non-incubated soils. In contrast, the number of root nodules t(8) = 7.65, p = 0.0001, were significantly higher for plants grown in soils incubated with 0.5% EPS.

Figure 5(a) shows the BEP plant shoots and roots after removal from the potted soil and immersed in deionized water. The root density was higher for plants grown in non-incubated soils than in soils incubated with 0.5% EPS. The root density in soils incubated with 0.1% EPS was higher than those in non-incubated soils (Figure 5(b)). Plant growth data showed that the average stem height, number of leaves per plant, root length, and root/shoot dry mass ratio increased with increase in EPS concentrations, although not significantly. However, there was a significant increase in leaf area, number of root nodules per plant, shoot biomass, and root biomass for bean plants grown in soils incubated with 0.1% EPS compared with plants grown in non-incubated soils. There was no significant difference in the measured plant growth parameters of plants grown in soils incubated with 0.02% and 0.10% (w/w) EPS (Table 5). Generally, EPS delayed plant germination in the soils. Incubating soils with EPS increased the vegetative growth of plant at low EPS concentrations (0.1% w/w). At higher




(a)




(b)

Figure 5. (a) Plant shoots and roots 31 days after seed sowing the roots immersed in deionized water. (b) Plant root development in various EPS incubated soils.

**Table 5.** Plant growth parameters at 0%, 0.02%, and 0.10% EPS concentrations in soil. Values are means (with standard deviations) of stem height (SH), number of leaves per plant (LPP), leaf area per plant (LA), root length (RL), number of root nodules per plant (RN), shoot biomass (SBM), root biomass (RBM), and root/shoot dry mass ratio (RBM/SBM).

| Soil EPS | SH/cm | LPP | LA/cm$^2$ | RL/cm | RN | SBM/g dry mass | RBM/g dry mass | RBM/SBM |
|---|---|---|---|---|---|---|---|---|
| 0% | 18.82 ± 2.29$^a$ | 9.8 ± 1.64$^a$ | 148.78 ± 6.31$^b$ | 17.66 ± 3.06$^a$ | 4.8 ± 4.8$^b$ | 1.258 ± 0.121$^b$ | 0.275 ± 0.048$^b$ | 0.218 ± 0.020$^a$ |
| 0.02% | 19.60 ± 1.63$^a$ | 10.6 ± 1.52$^a$ | 161.64 ± 17.51$^{a,b}$ | 18.48 ± 3.70$^a$ | 20.0 ± 4.85$^a$ | 1.349 ± 0.107$^{ab}$ | 0.303 ± 0.027$^{a,b}$ | 0.225 ± 0.019$^a$ |
| 0.10% | 20.16 ± 2.66$^a$ | 11.6 ± 1.34$^a$ | 182.83 ± 11.56$^a$ | 21.54 ± 5.48$^a$ | 24.0 ± 9.72$^a$ | 1.425 ± 0.047$^a$ | 0.339 ± 0.009$^a$ | 0.238 ± 0.009$^a$ |

**Mean values with similar letter indicate not significantly different based on Tukey's Studentized Range (HSD) test at $\alpha = 0.05$.

EPS concentrations (0.5% w/w) plant growth performance decreased compared with plants grown in non-incubated soils.

### 3.3. Evaluation of *R. tropici*-Derived Extracellular Polymeric Substances and Plant Growth on Soil Microbiota

The growth of Black-eyed peas (BEP) in EPS-incubated and non-incubated soils on soil microbial biomass and soil microbiota communities were evaluated using the PLFA analysis techniques. It was observed that incubating soils with or without EPS significantly increased the soil microbial biomass (total PLFA). Total microbial biomass was significantly higher in soils incubated with 0.1% EPS than in soils incubated with 0.5% EPS. Fungi biomass was higher in soils with 0.5% EPS, while the biomass of the other microbial groups was higher in soils incubated with 0.1% EPS (Table 6). Growing BEP in non-incubated soils increased the soil microbial biomass due to an increase in the biomass of Gram (−ve) bacteria, arbuscular mycorrhizal fungi, fungi, and actinomycetes; however, there was a decrease in Gram (+ve) biomass (Table 6). Growing BEP in EPS-incubated soils increased the soil microbial biomass due to increase in the arbuscular mycorrhizal fungi, Gram (+ve) bacteria, and fungi. The biomass of Gram (−ve) bacteria and actinomycetes increased with 0.1% EPS but decreased with 0.5% EPS. Generally, EPS significantly increased soil microbial biomass and shifted microbial populations from bacteria to fungi and from Gram (−ve) to Gram (+ve) bacteria after 30 days. On the contrary, the plant growth shifted the microbial population from Gram (+ve) to Gram (−ve) bacteria. Whereas 0.1% EPS greatly increased the Gram (+ve)/Gram (−ve) ratio compared to 0.5% EPS, the 0.5% EPS; increased the F/B ratio compared to the 0.1% EPS.

### 3.4. Evaluation of *R. tropici*-Derived Extracellular Polymeric Substances on Soil Chemical and Physical Properties

The water loss from the soils used in growing BEP was monitored for the initial 8 days before the foliar leaves appeared. Results showed that moisture loss from EPS-incubated soils was less than that of the non-incubated soils (Figure 6). Results showed that during the hot days in the greenhouse, the plants growing in non-incubated soils folded their leaves upwards earlier than plants growing in

**Table 6.** *R. tropici*-derived extracellular polymeric substances (EPS) and plant growth on soil microbial biomass and microbial composition.

| Soil amendment | Microbial biomass (pmol/g soil) | | | | | | | | Microbial biomass ratios | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total PLFA | Total NLFA | Fungi | AM Fungi | GN | GP | Eukary | Actino | F/B | GP/GN | GN stress | Total NLFA/ PLFA |
| Control | 57454.6 | 85060.8 | 402.5 | 1560.5 | 17449.2 | 13010.3 | 472.1 | 8263.5 | 0.0647$^{f}$ | 1.219$^{c}$ | 0.634$^{d}$ | 3.303$^{d}$ |
| 0.1% EPS | 66973.4 | 53963 | 447.6 | 1895.8 | 20324.7 | 15655.4 | 733.7 | 9908.5 | 0.0658$^{e}$ | 1.257$^{a}$ | 0.664$^{b}$ | 5.218$^{a}$ |
| | (16.6%) | (−36.6%) | (11.2%) | (21.5%) | (16.5%) | (20.3% | (55.4%) | (19.9%) | | | | |
| 0.5% EPS | 66158.2 | 50758.9 | 463.7 | 1950.4 | 19496.3 | 15108.1 | 578.5 | 9209.8 | 0.1072$^{a}$ | 1.247$^{b}$ | 0.710$^{a}$ | 3.041$^{e}$ |
| | (15.1%) | (−40.3%) | (15.2%) | (25%) | (11.7%) | (16.1%) | (22.5%) | (11.4%) | | | | |
| BEP | 58050.6 | 47167.6 | 439.4 | 1661.6 | 18430.3 | 12709.9 | 370.3 | 8302 | 0.0679$^{d}$ | 1.14$^{f}$ | 0.659$^{c}$ | 3.539$^{c}$ |
| | (1.0%) | (−44.5%) | (2.1%) | (6.5%) | (5.6%) | (−2.3%) | (−21.6%) | (0.5%) | | | | |
| BEP + 0.1% EPS | 62086.3 | 45738.8 | 481.1 | 1807.8 | 19116.9 | 13836 | 523 | 8848.1 | 0.0699$^{c}$ | 1.186$^{e}$ | 0.619$^{e}$ | 3.665$^{b}$ |
| | (8.1%) | (−46.2%) | (17.9%) | (15.8%) | (9.6%) | (6.3%) | (10.8%) | (7.1%) | | | | |
| BEP + 0.5% EPS | 58557.6 | 46420.9 | 501.9 | 1787.5 | 17707.8 | 13782.6 | 558.3 | 7681.3 | 0.073$^{b}$ | 1.212$^{d}$ | 0.586$^{f}$ | 2.488$^{f}$ |
| | (1.9%) | (−45.4%) | (20.7%) | (14.5%) | (1.5%) | (5.9%) | (18.3%) | (−7.0%) | | | | |
| CV | 6.8 | 27.6 | 78.4 | 8.2 | 5.9 | 8.2 | 22.4 | 9.1 | | | | |

**Mean values with similar letter indicate not significantly different based on Tukey's Studentized Range (HSD) test at $\alpha = 0.05$.

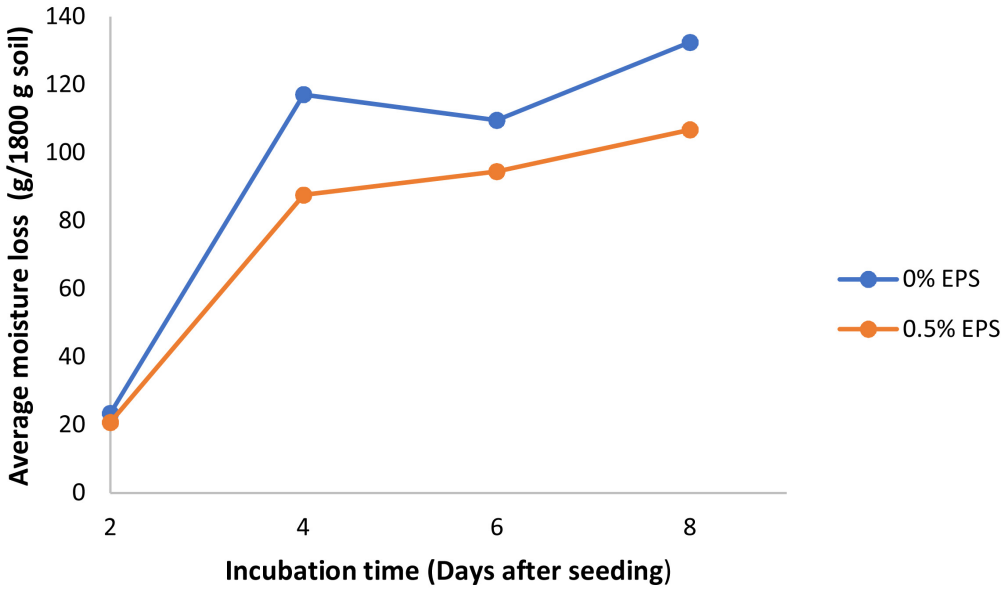



**Figure 6.** Relationship between EPS and soil water loss after 8 days of incubation.

EPS-incubated soils, in response to moisture stress (Figure 7). The results indicated that incubating the soils with *R. tropici* EPS significantly increased the soil aggregate stability, POC, and PMN. However, EPS had little or no effects on soil pH, soil organic matter (SOM), and cation exchange capacity (CEC) (Table 7).

**Figure 7.** Response of plants to moisture stress during hot temperatures in the greenhouse. Plants growing in 0% EPS (right) folded their leaves upwards to reduce water loss, earlier than plants in 5% EPS (Left).

**Table 7.** *Rhizobium tropici* EPS on soil chemical and physical properties.

| Soil amendment | $NH_4OAc$ Extractable Bases (meq/100g soil) | | | | | CEC (meq/100g soil) | % Base Saturation | SOM (%) | pH | | PMN | POC (mg/kg soil) | Aggregate Stability (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ca | Mg | Na | K | Sum | | | | $CaCl_2$ | $H_2O$ | | | |
| 0% EPS | 5.8 | 0.9 | 0.0 | 0.4 | 7.1 | 14.05 | 50.5 | 4.0 | 5.0 | 5.4 | 17.5 | 278.6 | 4.0 |
| | ±0.01[a] | ±0.0[a] | ±0.0[c] | ±0.0[a] | ±0.01[a] | ±0.01[a] | ±0.01[a] | ±0.07[a] | ±0.02[a] | ±0.03[a] | ±0.36[c] | ±0.72[c] | ±0.4[b] |
| 0.1% EPS | 5.7 | 0.9 | 0.03 | 0.4 | 7.1 | 14.3 | 49.7 | 3.97 | 5.0 | 5.4 | 18.5 | 310.6 | 17.0 |
| | ±0.02[a] | ±0.0[a] | ±0.0[b] | ±0.0[a] | ±0.01[a] | ±0.02[a] | ±0.04[a] | ±0.04[a] | ±0.02[a] | ±0.03[a] | ±0.34[b] | ±0.6[b] | ±1.1[a] |
| 0.5% EPS | 5.8 | 0.9 | 0.06 | 0.4 | 7.1 | 13.8 | 51.7 | 4.0 | 5.0 | 5.4 | 19.8 | 411.7 | 18.7 |
| | ±0.03[a] | ±0.0[a] | ±0.0[c] | ±0.0[a] | ±0.01[a] | ±0.03[a] | ±0.05[a] | ±0.04[a] | ±0.02[a] | ±0.03[a] | ±0.34[a] | ±0.80[a] | ±1.1[a] |

*Values (±standard deviation) with similar letters indicate not statistically and significantly different based on Tukey's Studentized Range (HSD) comparison with $\alpha = 0.05$.

## 4. Discussion

### 4.1. Evaluation of *R. tropici* EPS on Soil Respiration

Incubating soils with EPS increased soil respiration, suggestive of higher biological activities compared to the non-incubated soils. The increase in soil respiration was accompanied by an increase in soil microbial biomass (PLFA), in particular fungi and bacteria (Table 2, Figure 2). This is significant to soil health, fertility, plant growth, and development. Also, EPS serves as an energy source for soil microorganisms [27] which may account for higher biological activity and increased soil respiration. When EPS was combined with corn leaves the soil respiration increased tremendously. This combination will enhance soil management practices especially where EPS is used for growing cover crops to mitigate soil erosions. The EPS is reported to improve soil physical properties and reduce soil erosions [4] [9]. In the short term, higher soil respiration rates are not desirable because it may indicate the ease of decomposition of the EPS in soils which may limit their potential as a soil binder to strengthen earthen

structures [28] [29]. The easier the decomposition of EPS in soils, the less effective its soil strengthening capability with time.

### 4.2. Evaluation of *R. tropici*-Derived Extracellular Polymeric Substances on Seed Germination and Plant Growth

Results showed that EPS delayed the seeds germination which may be explained in part by the fact that when water evaporates from the surface of the potted soils, the water from below moves upwards by capillary action carrying the dissolved EPS. As the water evaporates the soil-EPS mixture left behind forms a hard concrete at the soil surface, preventing the seeds from sprouting. Although EPS delayed seeds germination in potted soils, the germinated seedlings had healthy growth; however, previous studies by Luo *et al*. [30] showed that the germinating seeds were faster in EPS solutions prepared with tap water. The plant growth parameters, stem height, number of leaves per plant, leaf area, and root length were higher in plants grown in non-incubated soils. Although at 0.5% EPS in soils plant root biomass was significantly reduced but the plant roots transfer large amounts of carbon to below-ground storage [30] [31]. At EPS-soil concentrations of ≤ 0.1% (w/w), the root biomass and vegetative growth of BEP in soils incubated with EPS were higher than for plants in soils without EPS. Similar results were obtained by Luo *et al*. [30] which showed that EPS enhanced bean plant biomass. The number of root nodules in plants growing in EPS-incubated soils was significantly greater than for plants growing in non-incubated soils (**Figure 4**, **Table 5**) suggesting that the EPS stimulates the formation of root nodules to provide the habitat for symbiotic nitrogen-fixing rhizobia bacteria in BEP. The average leaf area increased with an increase in EPS concentration (up to 0.10%) in the soils. An increase in leaf area may infer an increase in photosynthetic potentials of the plant, hence may explain in part the increase in plant biomass.

### 4.3. Evaluation of *R. tropici*-Derived Extracellular Polymeric Substances and Plant Growth on Soil Microbiota

The PLFA technique was used to determine the effects of EPS and plant growth on soil microbial biomass and microbial communities. While not specific like the DNA-based techniques, PLFA decomposes quickly upon cell death, so quantification of total PLFA in a soil samples represents the concentrations of living cells [29] [31]. The advantage of using PLFA over the PCR technique to estimate soil microbial community compositions is that extracellular DNA from dead microorganisms can persist in soils for weeks to years leading to the overestimation of the current microbiota [32]. Incubating soils with EPS did shift the microbial populations towards Gram (+ve) bacteria while growing the plant in the soils shifted the microbiota towards Gram (−ve) bacteria (**Table 5**). Gram (+ve) bacteria are associated with the decomposition of complex organic materials partially decomposed by fungi or Gram (−ve) bacteria [33] [34]. The increase in

Gram (+ve) bacteria biomass on the addition of EPS in soils may be indicative of these bacteria using EPS as a carbon source compared to Gram (−ve) bacteria. Gram (−ve) bacteria are involved in all phases of the nitrogen cycle and are considered significant in N-fixation by leguminous plants. It is likely therefore that the plant roots will attract the growth of Gram (−ve) bacteria. Extracellular polymeric substances also favored the growth of fungi, particularly Arbuscular mycorrhizae fungi. Arbuscular mycorrhizae fungi are known to be symbiotically related with over 67% of terrestrial plants for mineral nutrients acquisitions [35].

The increase in the plant growth parameters in soils incubated with 0.1% EPS was accompanied by an increase in soil microbial biomass compared to soils incubated with 0.5% EPS. This indicates that although 0.5% (w/w) EPS in soils may increase soil strengths and lower concentrations seemed more appropriate for the plant growth. Measurement of neutral lipid fatty acids (NLFAs) and phospholipid fatty acids (PLFAs) have been used to assess soil perturbations on soil microorganisms. Low NLFA/PLFA ratios have been attributed to fatty acids common in bacteria, especially cyclopropane fatty acids, while higher ratios have been attributed to fatty acids common in eukaryotic organisms such as fungi [36]. This study shows, 0.1% EPS in soil increased the NLFA/PLFA ratio while 0.5% EPS decreased the ratio compared to the control (**Table 2** and **Table 6**). The findings also indicate that both 0.1% and 0.5% EPS increased the ratio of monounsaturated fatty acids and cyclopropane fatty acids measured as the Gram (−ve) stress. When Gram-negative bacteria are under stress conditions in soils their membrane phospholipids change from monounsaturated fatty acids to cyclopropane fatty acids. Overall, the increase in this ratio (*i.e.* 16:1Ω7c + 18:1Ω7c)/(cy17:0 + cy19:0) indicates reduction in microbial stress [37] [38].

### 4.4. Evaluation of *R. tropici*-Derived Extracellular Polymeric Substances on Soil Chemical and Physical Properties

*R. tropici* derived EPS increased soil active carbon (POC). This may partially explain the increase in soil respiration in EPS-incubated soils. The increase in the soil potential nitrogen mineralization on adding EPS (**Table 7**) corresponded to an increase in the soil microbial biomass. Potential mineralizable nitrogen has been considered as an indicator of biologically active soil nitrogen, and a soil quality attribute [39]. Incubating the soils with EPS significantly increased the soil's aggregate stability (**Table 7**). This in part explains why the EPS has been used to strengthen earthen structures and to mitigate soil erosions. The effects of EPS on the soil properties suggest its potential as a soil enhancer for agricultural and geotechnical purposes. The slower rate of water loss from EPS-incubated soils compared to non-incubated soils (**Figure 6**) may enable plants to survive longer during periods of moisture stress. Our study showed that during the hot days in the greenhouse, the leaves of plants grown in the non-incubated soils folded upward earlier than plants growing in EPS-incubated soils in response to moisture stress (**Figure 7**).

## 5. Conclusion

Previous studies have been carried out for the industrial application of *R. tropici*-derived EPS and its use in improving soil properties and engineering structures. In this study, we established that EPS enhances soil biological, chemical, and physical properties by increasing soil respiration, microbial biomass, active carbon, potentially mineralizable nitrogen, and soil aggregate stability. Extracellular polymeric substances shifted the soil microbial population towards Gram (+ve) bacteria, while growing BEP shifted towards Gram (−ve) bacteria in the short term. At low EPS concentrations (equal to or less than 0.1%, w/w) in soils, the vegetative growth of the plant was higher than in non-incubated soils. At 0.5% EPS concentration in soils, the growth of the legume was lower than in the non-incubated soils. While 0.5% EPS (w/w) in soils may be more applicable in stabilizing and strengthening soils to mitigate erosions or strengthen geotechnical/engineering structures, lower concentrations may be more applicable to improve soil health and fertility indices. Studies on EPS to improve soil health and fertility indices, crop production, and engineering structures necessitate a multidisciplinary approach and longitudinal study over a long period.

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## Data Availability

Data will be made available on request.

## Declaration of Competing Interests

The authors declare that they have no competing interests or personal relationships that could have appeared to influence the work reported in this paper.

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