

**Evaluation of Plant Growth Promotion Effects of Coal-Processed Soil Conditioner:
Phase 2**

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Introduction

In the previous phase of this project, the effects of coal-derived soil amendments developed by Advanced Environmental Technologies (AETEC) on plant growth of lettuce and pepper and soil properties were evaluated. The application of Ginate in soils was found to promote both lettuce (*Lactuca sativa*) and pepper (*Capsicum annuum*) growth and resulted in significantly higher plant weight in comparison to the treatment of organic fertilizer. Furthermore, BGF1 at the same dosage had better effects on pepper plant growth and fruit productivity in comparison to the commercial organic fertilizer.

In the second phase of the project, CSU evaluated four additional coal-derived soil amendments (ECF type) on lettuce growth and selected soil properties. Several different tests were conducted to evaluate various ECF products, blends of ECF with Ginate, and Liquid Fertilizer on growth of lettuce. These tests are summarized in Table 1.

Table 1: Summary of Phase 2 tests using lettuce and various coal-derived soil amendments.

| Test | Purpose |
|-------------|---|
| 1 | Evaluate ECF-FM-0.5 and ECF-FM-1.0 |
| 2 | Evaluate BCF-FM-22A, BCF-FM-22B, BCF-FM-22C, BCF-FM-22D, BCF-FM-22E |
| 3 | Evaluate selected ECF products (fine powders) |
| 4 | Evaluate mixtures of ECF products and Ginate at different ratios |
| 5 | Evaluate selected ECF products mixed with Ginate at fixed ratio (51:49) at different dosages (0.4 – 1.6%) |
| 6 | Evaluate 8 new ECF products alone and mixed with Ginate |
| 7 | Evaluate the dosage effects of Ginate and compare to Liquid Fertilizer (both 2018 and 2019) |

Test 1

Purpose: To determine the effects of ECF-FM-0.5 and ECF-FM-1.0 with or without nitrogen on the plant growth of lettuce.

Experimental design: There were 9 treatments in this test as detailed in Table 2. Lettuce seedlings at the 2-true-leaves stage were transplanted into the treatment pots. All lettuce plants were grown under the same conditions during the test period in the greenhouse at Colorado State University. There were 6 plants for each treatment and 1 plant per pot. Plants were harvested at the end of 5 weeks.

Results: As shown in Figure 1, the lettuce plants at 5 weeks were larger in the treatments of 0.4% chemical, BGF1, BGF1+chemical, Ginate, and ECF-FM-1.0+chemical in comparison to the reference (soil without amendments) and ECF treatments without chemical. The ECF-FM-0.5+ treatment did not promote plant growth. Ginate exhibited the largest effect on plant weight and height. Neither ECF-FM-0.5 nor ECF-FM-1.0 had positive effects on lettuce growth.

Table 2: Summary of Test 1 treatments.

| Treatment# | Fertilizer Type | Fertilizer Amount, wt% | Nitrogen Blended to 7 wt% Solids (as is basis)? |
|-------------------|------------------------|-------------------------------|--|
| 1 | None | 0 | NA |
| 2 | Chemical | 0.4 | NA |
| 3 | BGF1 | 0.4 | N |
| 4 | BGF1+ | 0.4 | Y |
| 5 | Ginate (2020) | 0.4 | N |
| 6 | ECF-FM-0.5 | 1 | N |
| 7 | ECF-FM-0.5+ | 1 | Y |
| 8 | ECF-FM-1.0 | 1 | N |
| 9 | ECF-FM-1.0+ | 1 | Y |

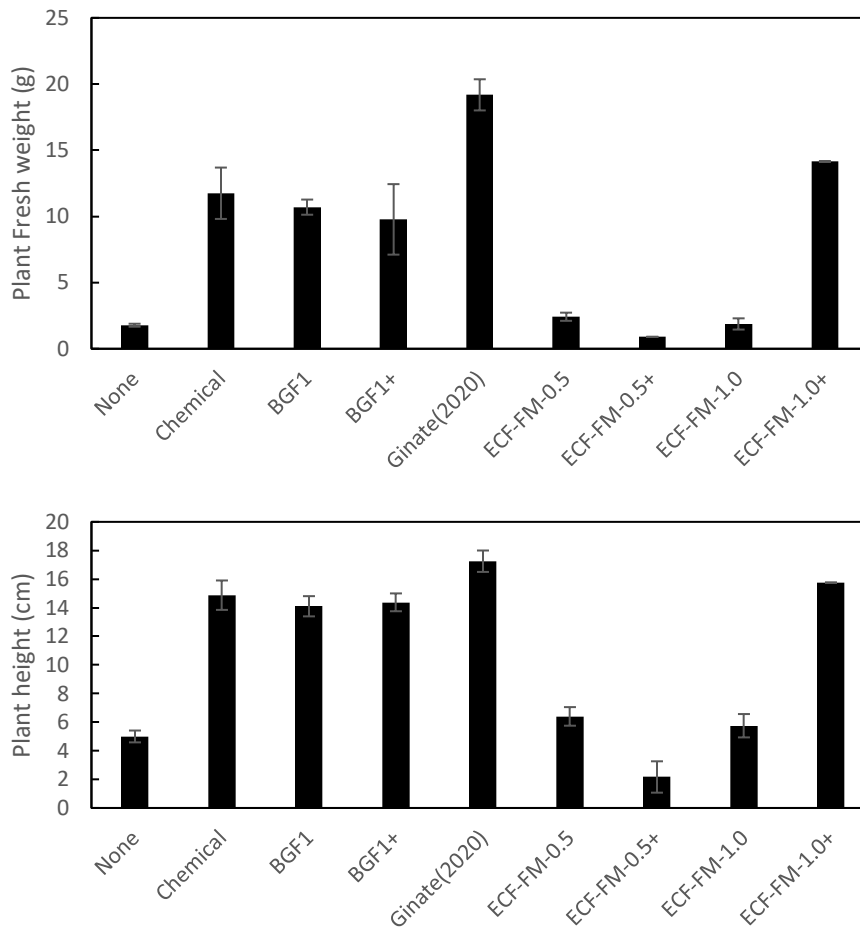


Figure 1. The fresh weight and height of lettuce plants with different treatments when harvested at 5 weeks after transferring. The values are the average of 6 plants of each treatment. The error bars represent one standard deviation of 6 biological replicates.

Test 2

Purpose: To determine the effects of a group of the ECF products BCF-FM-22A, BCF-FM-22B, BCF-FM-22C, BCF-FM-22D, and BCF-FM-22E on the growth of lettuce.

Experimental design: The 9 treatments in this test are outlined in Table 3. ECF products were mixed in the soil at 1.8% while BGF1, Ginate, and the commercial fertilizer were mixed in the soil at 0.4%. Lettuce seedlings were prepared in the same way as in Test 1. Lettuce seedlings at the 2-true-leaves stage were transplanted into the treatment pots. All lettuce plants were grown at the same conditions during the test period in the greenhouse at Colorado State University. There were 6 plants for each treatment and 1 plant per pot. Plants were harvested at the end of 5 weeks.

Results: The ECF products were newly prepared and thus had a high moisture content. We applied 1.8% wet weight in the soil which is like the 0.4% dry weight used for Ginate. As shown in Figure 2, lettuce plants of all the treatments of ECF products grew to a similar height and weight in comparison to the reference (without soil amendment). The treatments of BGF1 and Ginate-2020 (positive controls) exhibited significantly higher plant height and weight than those of the reference (soil without addition). These results indicate that the ECF products tested here did not have positive effects on lettuce growth.

Table 3: Summary of Test 2 treatments.

| Treatment | Fertilizer Type | Fertilizer Amount, wt% | Chemical Nitrogen Blended to 7% Solids (as is basis)? |
|------------------|------------------------|-------------------------------|--|
| 1 | None | 0 | NA |
| 2 | Commercial | 0.4 | N |
| 3 | BGF1 | 0.4 | N |
| 4 | Ginate-2020 | 0.4 | N |
| 5 | BCF-FM-22A | 1.8 | N |
| 6 | BCF-FM-22B | 1.8 | N |
| 7 | BCF-FM-22C | 1.8 | N |
| 8 | BCF-FM-22D | 1.8 | N |
| 9 | BCF-FM-22E | 1.8 | N |

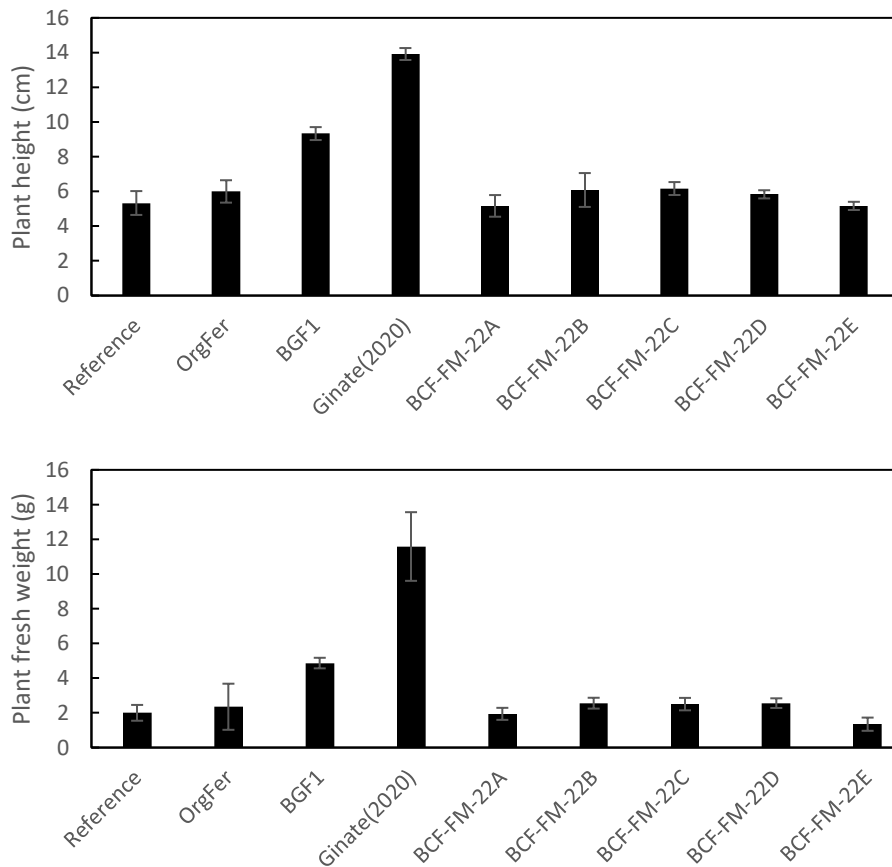


Figure 2. The fresh weight and height of lettuce plants with different treatments when harvested at 5 weeks after transferring. The values are the average of 6 plants of each treatment. Error bars represent one standard deviation of the 6 biological replicates.

Test 3

Purpose: In Tests 1 and 2, none of the ECF products had a positive effect on lettuce growth. The particle size in the products may play an important role in promoting plant growth. In this test, selected ECF products were ground to fine powders to match the size of the Ginate product, then mixed in soils at the same amount as Ginate to determine the effects on the growth of lettuce.

Experimental design: There were 9 treatments in this test as outlined Table 4. All the soil amendments were mixed in the soil at 0.4% (w/w). Lettuce seedlings were prepared in the same way as in Test 1. There were 6 plants for each treatment and 1 plant per pot. Plants were harvested at the end of 5 weeks.

Results: As shown in Figure 3, none of the treatments had a significant effect on the growth of

lettuce, except Ginate-2020. Therefore, the particle size did not affect the effects of these newly developed ECFs.

Table 4: Summary of Test 3 treatments.

| Treatment# | Fertilizer Type | Fertilizer Amount, % |
|-------------------|------------------------|-----------------------------|
| 1 | None | 0 |
| 2 | Chemical | 0.4 |
| 3 | Ginate-2020 | 0.4 |
| 4 | BCF-FM-14B | 0.4 |
| 5 | BCF-FM-14C | 0.4 |
| 6 | BCF-FM-22A | 0.4 |
| 7 | BCF-FM-22D | 0.4 |
| 8 | ECF-FM-0.5 | 0.4 |
| 9 | ECF-FM-1.0 | 0.4 |

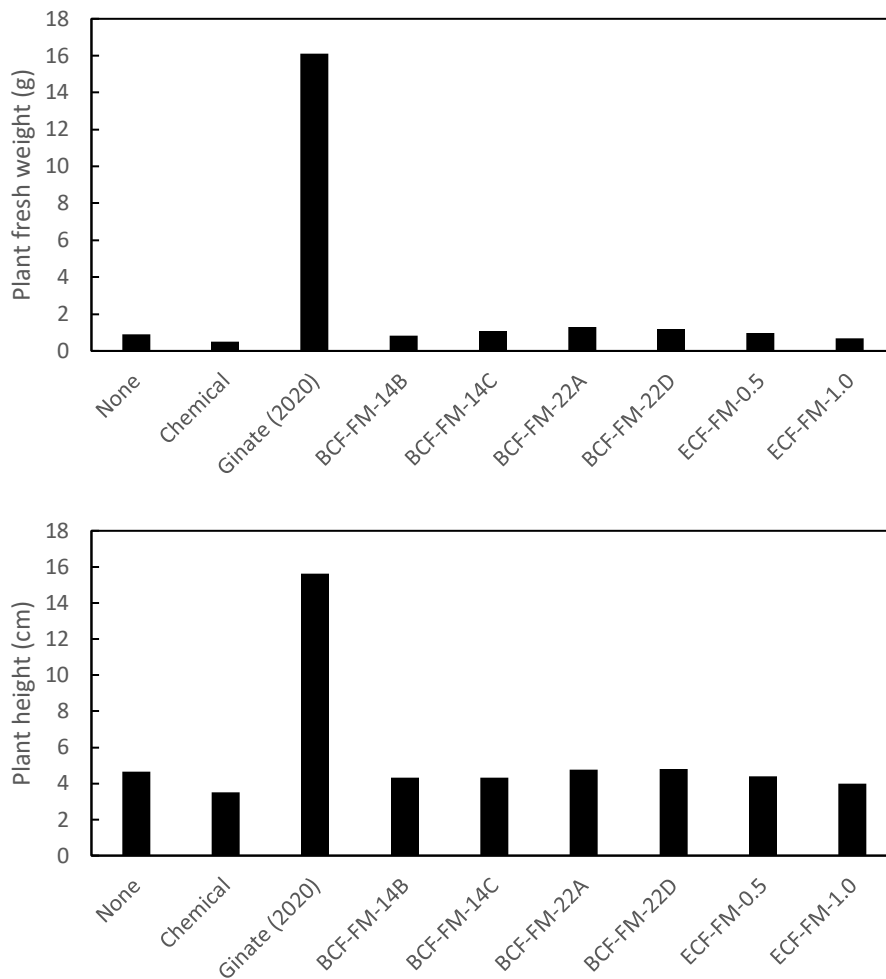


Figure 3. The fresh weight and height of lettuce plants with different treatments when harvested at 5 weeks after transferring. The values are the average of 6 plants of each treatment.

Test 4

Purpose: To evaluate the effect on lettuce growth of mixing selected ECF products with Ginate at different ratios.

Experimental design: As outlined in Table 5 , there were 15 treatments in this test. BCF-FM-22A and ECF-FM-1.0 were mixed with Ginate at 80:20 and 60:40, respectively. All soil amendments were mixed in the soil at either 0.4 or 0.8 wt%. Lettuce seedlings were prepared in the same way as described above. There were 6 plants for each treatment and 1 plant per pot. Plants were

harvested after 5 weeks.

Results: As shown in Figure 4, Ginate-2020 at both 0.4 and 0.8 wt% had significant effects on promoting the growth of lettuce. ECF1-1 (BCF-FM-22A) and ECF (ECF-FM-1.0) at both 0.4 and 0.8% did not promote plant growth. The mix of Ginate and ECF products at different ratios resulted in higher plant height and weight in comparison to BCF-FM-22A, ECF-FM-1.0, and reference. These results indicate the blends of Ginate and ECF products can promote plant growth. Ginate appears to be the major contributor to plant growth. Therefore, the dosage of application of mix products need to be optimized.

Table 5: Summary of Test 4 treatments.

| Treatment ID | ECF | BGF | wt% ECF | wt% BGF | Mix in soil (wt%) |
|---------------------|------------|-------------|----------------|----------------|--------------------------|
| REF | NA | NA | 0 | 0 | |
| BGF-1 | NA | Ginate-2020 | 0 | 100 | 0.4 |
| BGF-2 | NA | Ginate-2020 | 0 | 100 | 0.8 |
| ECF1-1 | BCF-FM-22A | NA | 100 | 0 | 0.4 |
| ECF1-2 | BCF-FM-22A | NA | 100 | 0 | 0.8 |
| EB2-1 | BCF-FM-22A | Ginate-2020 | 80 | 20 | 0.4 |
| EB2-2 | BCF-FM-22A | Ginate-2020 | 80 | 20 | 0.8 |
| EB4-1 | BCF-FM-22A | Ginate-2020 | 60 | 40 | 0.4 |
| EB4-2 | BCF-FM-22A | Ginate-2020 | 60 | 40 | 0.8 |
| ECF | ECF-FM-1.0 | NA | 100 | 0 | 0.4 |
| ECF | ECF-FM-1.0 | NA | 100 | 0 | 0.8 |
| EB6-1 | ECF-FM-1.0 | Ginate-2020 | 80 | 20 | 0.4 |
| EB6-2 | ECF-FM-1.0 | Ginate-2020 | 80 | 20 | 0.8 |
| EB8-1 | ECF-FM-1.0 | Ginate-2020 | 60 | 40 | 0.4 |
| EB8-2 | ECF-FM-1.0 | Ginate-2020 | 60 | 40 | 0.8 |

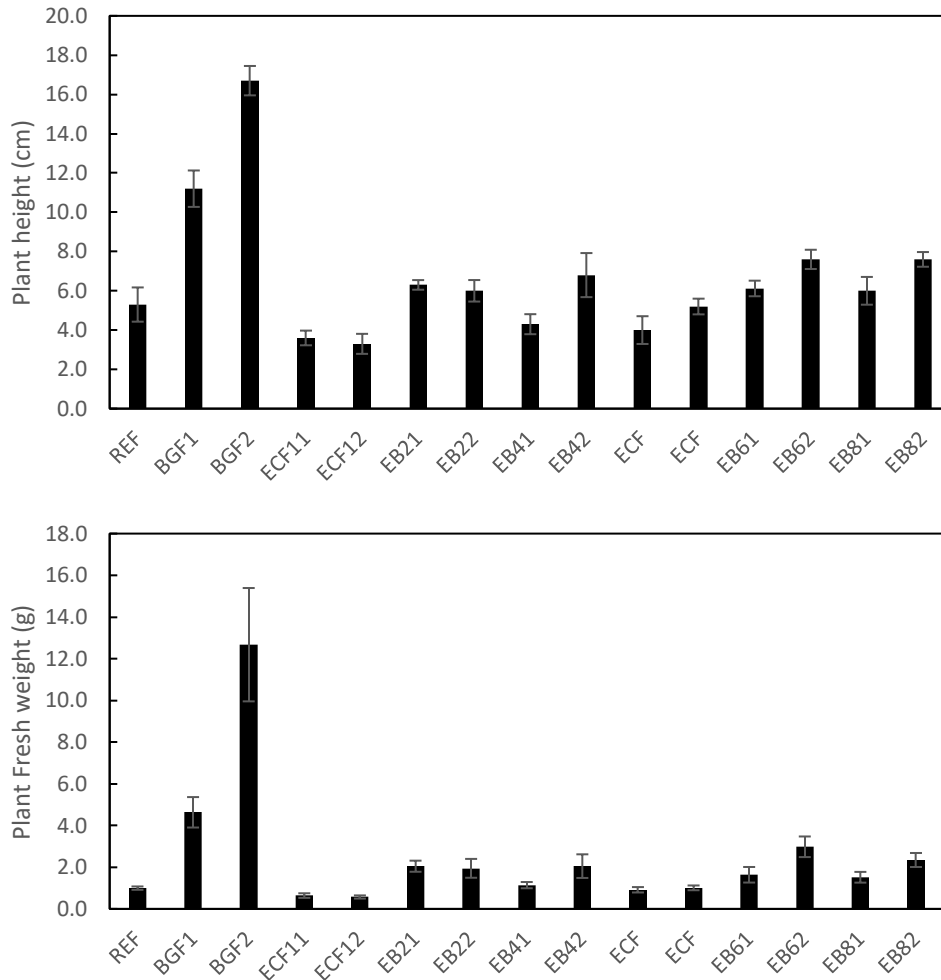


Figure 4. The fresh weight and height of lettuce plants with different treatments when harvested at 5 weeks after transferring. The values are the average of 6 plants of each treatment. The error bar represents the standard deviation of the 6 biological replicates.

Test 5

Purpose: In Test 4, the blends of ECF products with Ginate showed positive effects on the growth of lettuce. In this test, the selected ECF products were mixed with Ginate at fixed ratio (51:49), then applied in soils at different dosages ranging from 0.4 to 1.6% to determine the effects on the plant growth of lettuce.

Experimental design: There were 17 treatments in this test (Table 6). Both BCF-FM-22A and ECF-FM-1.0 were mixed with Ginate at the ratio of 51:49, respectively. Then different amounts of the mixture (0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6% w/w) were added to soils that were then put in

pots. Lettuce seedlings were prepared in the same way as in previous experiments. There were 6 plants for each treatment and 1 plant per pot. Plants were harvested at the end of 5 weeks.

Results: First, Ginate-2020 at both 0.4% and 0.8% showed significant positive effects on the growth of lettuce while neither BCF-FM-22A nor ECF-FM-1.0 alone had an effect (Figure 5). The blends of Ginate and ECF products exhibited higher plant height and weight in comparison to BCF-FM-22A, ECF-FM-1.0, and reference. Furthermore, the application of blends (Ginate+BCF-FM-22A) at higher amount resulted in higher plant height and weight from 0.4 to 0.8%. Higher application above 0.8% (from 0.8% to 1.6%) did not result in significant higher plant height and weight than 0.8% blends. The application of blends (Ginate+ECF-FM-1.0) had a similar pattern on promoting plant growth as the blend Ginate+BCF-FM-22A.

These results indicate the blends of Ginate and ECF products at the ratio 51:49 can promote plant growth and the application dosage should be 0.8% (w/w) or higher to ensure similar effects as Ginate at 0.4%.

Table 6: Summary of Test 5 treatments.

| Label | Treatment ID | ECF | BGF | wt% ECF | Wt% BGF | Mix in soil (wt%) |
|--------------|---------------------|------------|-------------|--------------------|--------------------|------------------------------|
| BT-1 | REF | NA | NA | 0 | 0 | |
| BT-2 | BGF-0.4 | NA | Ginate-2020 | 0 | 100 | 0.4 |
| BT-3 | BGF-0.8 | NA | Ginate-2020 | 0 | 100 | 0.8 |
| BT-4 | ECF1+BGF-0.4 | BCF-FM-22A | Ginate-2020 | 51 | 49 | 0.4 |
| BT-5 | ECF1+BGF-0.6 | BCF-FM-22A | Ginate-2020 | 51 | 49 | 0.6 |
| BT-6 | ECF1+BGF-0.8 | BCF-FM-22A | Ginate-2020 | 51 | 49 | 0.8 |
| BT-7 | ECF1+BGF-1.0 | BCF-FM-22A | Ginate-2020 | 51 | 49 | 1.0 |
| BT-8 | ECF1+BGF-1.2 | BCF-FM-22A | Ginate-2020 | 51 | 49 | 1.2 |
| BT-9 | ECF1+BGF-1.4 | BCF-FM-22A | Ginate-2020 | 51 | 49 | 1.4 |
| BT-10 | ECF1+BGF-1.6 | BCF-FM-22A | Ginate-2020 | 51 | 49 | 1.6 |
| BT-11 | ECF2+BGF-0.4 | ECF-FM-1.0 | Ginate-2020 | 51 | 49 | 0.4 |
| BT-12 | ECF2+BGF-0.6 | ECF-FM-1.0 | Ginate-2020 | 51 | 49 | 0.6 |
| BT-13 | ECF2+BGF-0.8 | ECF-FM-1.0 | Ginate-2020 | 51 | 49 | 0.8 |
| BT-14 | ECF2+BGF-1.0 | ECF-FM-1.0 | Ginate-2020 | 51 | 49 | 1.0 |
| BT-15 | ECF2+BGF-1.2 | ECF-FM-1.0 | Ginate-2020 | 51 | 49 | 1.2 |
| BT-16 | ECF2+BGF-1.4 | ECF-FM-1.0 | Ginate-2020 | 51 | 49 | 1.4 |
| BT-17 | ECF2+BGF-1.6 | ECF-FM-1.1 | Ginate-2020 | 51 | 49 | 1.6 |

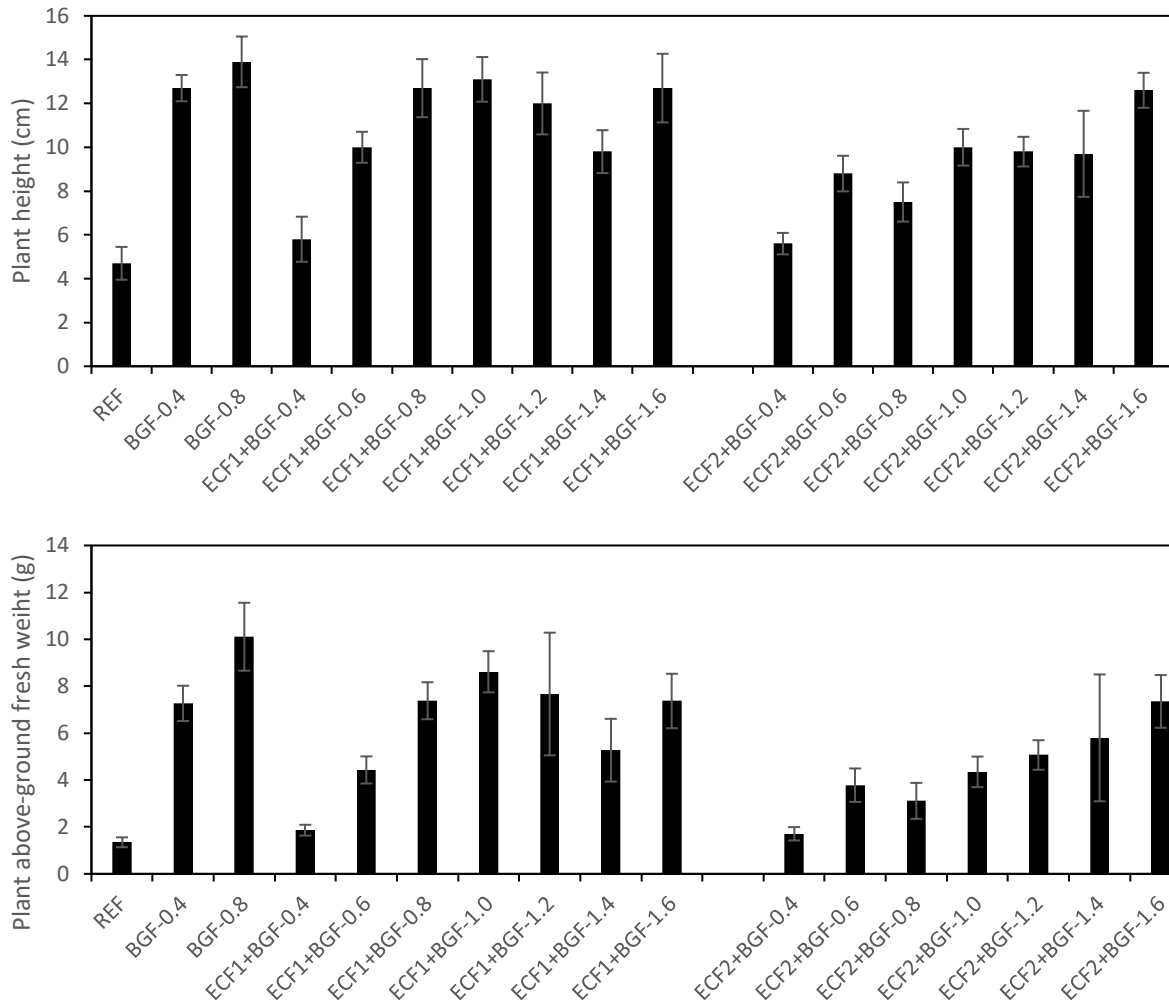


Figure 5. The fresh weight and height of lettuce plants with different treatments when harvested at 5 weeks after transferring. The values are the average of 6 plants of each treatment. The error bar represents the standard deviation of the 6 biological replicates.

Test 6

Purpose: In this test, 8 different new ECF products will be tested alone or mixed with Ginate to determine the effects on the plant growth of lettuce.

Experimental design: There were 18 treatments in this test as in the below table. Eight ECF products (FM-I, FM-II, FM-III, FM-IV, PRB-I, PRB-II, PRB-III, and PRB-IV) either applied alone (100%) at 0.4% w/w, or mixed with Ginate (50:50) and applied at 0.8% in soils. Lettuce seedlings were prepared in the same way as above mentioned. There were 6 plants for each treatment and 1 plant per pot. Plants were harvested at the end of 5 weeks.

Results: In comparison to the reference treatment, all the ECFs with or without Ginate-2020 showed significant effects on promoting the plant growth of lettuce, except FM-II-0.4 and PRB-IV-0.4 (Figure 6). The blends of Ginate and ECF products showed significant higher plant height and weight in comparison to ECF products alone, except FM-II-0.8 and PRB-IV-0.8. Furthermore, the application of blends at 0.8% w/w had similar plant height and weight to the Ginate-0.4, except PRB-IV-0.8. These results indicate the new ECF products can promote plant growth significantly, although the effects are not as good as Ginate applied at the same amount.

Table 7: Summary of Test 6 treatments.

| Treatment# | ECF ID | ECF, mix% | Ginate, mix% | Total Amount in Soil, wt% |
|-------------------|---------------|------------------|---------------------|----------------------------------|
| FPA-1 | None | 0 | 0 | 0 |
| FPA-2 | None | 0 | 100 | 0.4 |
| FPA-3 | FM-I | 100 | 0 | 0.4 |
| FPA-4 | FM-I | 50 | 50 | 0.8 |
| FPA-5 | FM-II | 100 | 0 | 0.4 |
| FPA-6 | FM-II | 50 | 50 | 0.8 |
| FPA-7 | FM-III | 100 | 0 | 0.4 |
| FPA-8 | FM-III | 50 | 50 | 0.8 |
| FPA-9 | FM-IV | 100 | 0 | 0.4 |
| FPA-10 | FM-IV | 50 | 50 | 0.8 |
| FPA-11 | PRB-I | 100 | 0 | 0.4 |
| FPA-12 | PRB-I | 50 | 50 | 0.8 |
| FPA-13 | PRB-II | 100 | 0 | 0.4 |
| FPA-14 | PRB-II | 50 | 50 | 0.8 |
| FPA-15 | PRB-III | 100 | 0 | 0.4 |
| FPA-16 | PRB-III | 50 | 50 | 0.8 |
| FPA-17 | PRB-IV | 100 | 0 | 0.4 |
| FPA-18 | PRB-IV | 50 | 50 | 0.8 |

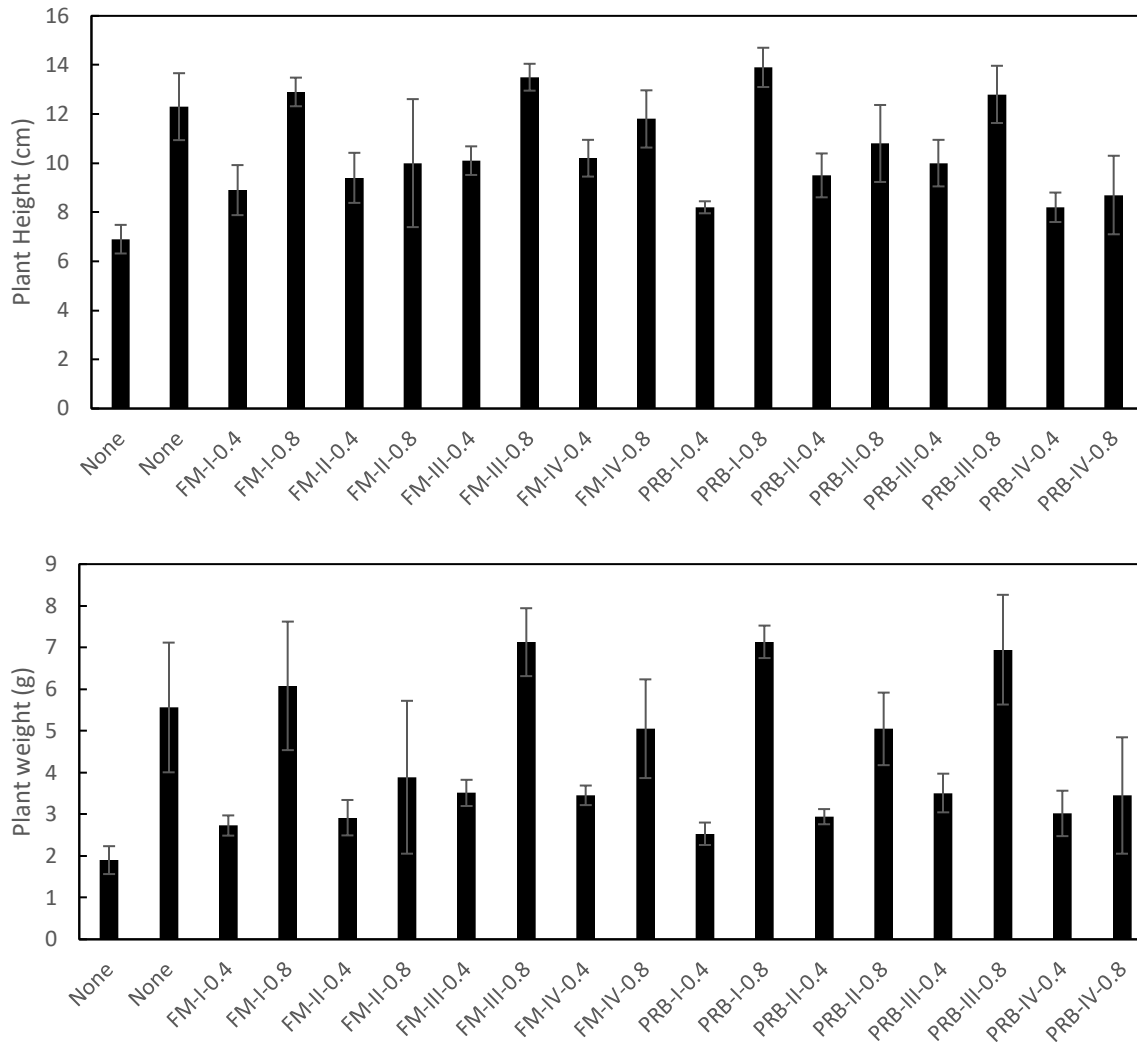


Figure 6. The fresh weight and height of lettuce plants with different treatments when harvested at 5 weeks after transferring. The values are the average of 6 plants of each treatment. The error bar represents the standard deviation of the 6 biological replicates.

Test 7

Purpose: In this test, two sets of treatments were conducted. In the first set, different amounts of Ginate-2020 were mixed in the soils to determine what the minimum application amount is to promote plant growth of lettuce. In the other set, different amount of Liquid Fertilizer (both 2018 and 2019) was added in the soils mixed with Ginate-2020 at 0.1% and 0.2% to determine the effects of Liquid Fertilizer on the plant growth of lettuce.

Experimental design: There were 18 treatments in this test (Table 8). In one set of treatments,

Ginate was mixed in the soil at 0.05, 0.1, 0.2, and 0.4% (w/w). In a second set, Liquid Fertilizers 2018 and 2019 were added in the Ginate treatments at 0.1% and 0.2% (w/w) at different amounts (A: 15, B: 30, and C: 45 mL per pot). Lettuce seedlings were prepared in the same way as described previously. There were 6 plants for each treatment and 1 plant per pot. Plants were harvested at the end of 5 weeks.

Results: The addition of Ginate alone in soil resulted in significantly higher plant height and weight at 0.2 and 0.4% (Figure 7). It indicates the minimum application of Ginate should be higher than 0.2% to promote lettuce growth.

In the tests with blends of Ginate and Liquid Fertilizers, the Liquid Fertilizer 2018 at different dosages promoted plant growth in both 0.1% and 0.2% Ginate while Liquid Fertilizer 2019 did not (Figure 8). Furthermore, the application of Liquid Fertilizer 2018 at different dosages showed that 45 mL had the strongest effects on promoting lettuce growth than 30 mL and 15 mL. These results indicate the Liquid Fertilizer 2018 promoted plant growth and the combination of Liquid Fertilizer 2018 with Ginate works better than application of them individually at the same amount.

Table 8: Summary of Test 7 treatments.

| Treatment ID | Ginate-2020 | Liquid Fertilizer | Liquid Fertilizer (mL) |
|---------------------|--------------------|--------------------------|-------------------------------|
| 1 | BGF 0.1% | 2018 Liquid Fertilizer | 0 |
| 2 | BGF 0.1% | 2018 Liquid Fertilizer | 15 |
| 3 | BGF 0.1% | 2018 Liquid Fertilizer | 30 |
| 4 | BGF 0.1% | 2018 Liquid Fertilizer | 45 |
| 5 | BGF 0.1% | 2019 Liquid Fertilizer | 15 |
| 6 | BGF 0.1% | 2019 Liquid Fertilizer | 30 |
| 7 | BGF 0.1% | 2019 Liquid Fertilizer | 45 |
| 8 | BGF 0.2% | 2018 Liquid Fertilizer | 0 |
| 9 | BGF 0.2% | 2018 Liquid Fertilizer | 15 |
| 10 | BGF 0.2% | 2018 Liquid Fertilizer | 30 |
| 11 | BGF 0.2% | 2018 Liquid Fertilizer | 45 |
| 12 | BGF 0.2% | 2019 Liquid Fertilizer | 15 |
| 13 | BGF 0.2% | 2019 Liquid Fertilizer | 30 |
| 14 | BGF 0.2% | 2019 Liquid Fertilizer | 45 |
| 15 | BGF 0.05% | - | - |
| 16 | BGF 0.1% | - | - |
| 17 | BGF 0.2% | - | - |
| 18 | BGF 0.4% | - | - |

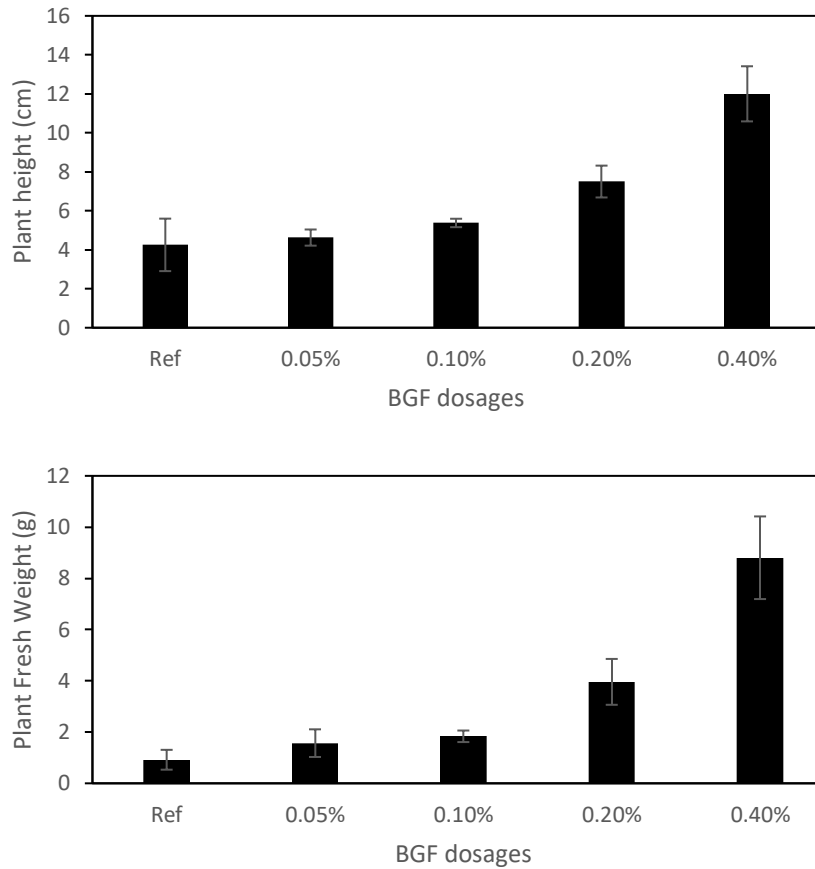


Figure 7. The fresh weight and height of lettuce plants with different treatments when harvested at 5 weeks after transferring. The values are the average of 6 plants of each treatment. The error bar represents the standard deviation of the 6 biological replicates.

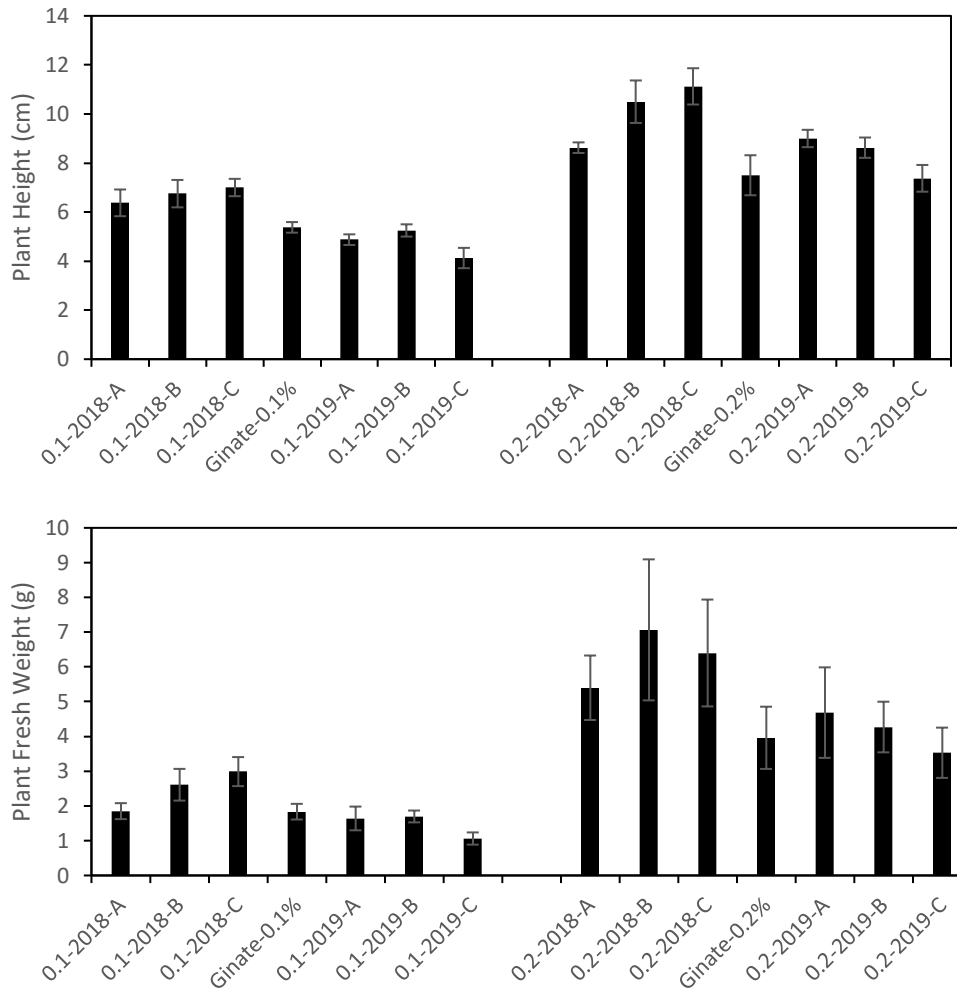


Figure 8. The fresh weight and height of lettuce plants with different treatments when harvested at 5 weeks after transferring. The values are the average of 6 plants of each treatment. The error bar represents the standard deviation of the 6 biological replicates.

Summary

Different preparations of ECF products led to different effects on lettuce growth. For instance, the ECF products in Tests 1–3 did not promote plant growth while different preparations of ECF products in Test 6 had positive effects. Moreover, blends of ECF products with Ginate were evaluated and had positive effects on plant growth depending on the application dosages. The application of Ginate should be 0.2% (w/w) or more to promote lettuce growth, and Liquid Fertilizer 2018 promoted plant growth. The combination of Liquid Fertilizer 2018 with Ginate had better effects than application of them individually at the same amount.