

## **Evaluation of Growth Promotion Effects of Coal-Processed Soil Conditioner: Phase 3**

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### **Introduction**

The effects of coal-derived soil amendments developed by Advanced Environmental Technologies (AETEC) on growth of lettuce and pepper and soil properties were evaluated in Phase 1. It was found that the application of Ginate in soils promoted both lettuce (*Lactuca sativa*) and pepper growth and resulted in higher plant biomass weight in comparison to the treatment of organic fertilizer. In Phase 2 of the project, seven tests were conducted to evaluate several ECF products, blends of ECF with Ginate, and Liquid Fertilizer on growth of lettuce. It was found that the preparation method of the ECF products resulted in different effects on the growth of lettuce. Moreover, blends of ECF products with Ginate were evaluated and found to have positive effects on growth depending on the application dosage.

The goal of this phase of the project was to determine the effects of Ginate application on the growth of hemp and on the root system development of lettuce, sweet pepper, and grass.

### **Effects of Ginate on hemp growth**

#### *Purpose*

To determine the effects of Ginate on hemp growth and to evaluate how much nutrient solution addition could be reduced when Ginate is applied in the soil.

#### *Experimental design*

In total, there were 15 treatments in this test as shown in Table 1. Ginate was mixed in the soil at 0%, 0.1%, and 0.4% (w/w). Hemp (variety: Working Wife) seeds were sowed in 1 gallon pots filled

with soils mixed with different amount of Ginate. There were 6 plants for each treatment and 1 plant per pot. During the growth period, nutrient solution at different strength (0, 30, 50, 80, and 100%) and water were applied to each pot as shown Table 1 every 3 days. All hemp plants were grown at the same conditions during the test period in the greenhouse at Colorado State University. The growth was monitored at 31 and 50 days after seeds were sown.

Table 1. Experimental design for hemp study.

<b>Treatment Number</b>	<b>Treatment Code</b>	<b>Solid Fertilizer</b>	<b>Fertilizer Dosage (%)</b>	<b>Nutrient Solution (%)</b>
1	GN0-100	None	0	100
2	GN0-80	None	0	80
3	GN0-50	None	0	50
4	GN0-30	None	0	30
5	GN0-0	None	0	0
6	GN0.1-100	Ginate	0.1	100
7	GN0.1-80	Ginate	0.1	80
8	GN0.1-50	Ginate	0.1	50
9	GN0.1-30	Ginate	0.1	30
10	GN0.1-0	Ginate	0.1	0
11	GN0.4-100	Ginate	0.4	100
12	GN0.4-80	Ginate	0.4	80
13	GN0.4-50	Ginate	0.4	50
14	GN0.4-30	Ginate	0.4	30
15	GN0.4-0	Ginate	0.4	0

### *Results*

As shown in Figure 1, the height of hemp plants at 31 days increased from 31 to 39 cm in the GN0 treatments and 34 to 42 cm in the GN0.1 treatments when the nutrient solution amount is increased from 0 to 100%. In the GN0.4 treatments, plant height was similar (about 40 cm) when nutrient solution amount was applied from 30 to 100% and was higher than that of the plants without nutrient solution (GN0.4-0, 35 cm). The change in the number of plant leaves followed a similar pattern as plant height in the different treatments.

These results indicate that Ginate at both 0.1 and 0.4% had positive effects on hemp growth.

Ginate at 0.4% had better effects than 0.1% as plant height was similar when less nutrient solution (as low as 30%) was applied.

After 50 days, the growth of hemp plants followed a similar pattern as was observed at 31 days. As shown in Figure 2, the height of hemp plants at 50 days in the GN0 treatments was significantly higher when the nutrient solution amount was above 80% than when it was at 50% and below. The height of hemp plants increased from 48 to 65 cm in the GN0.1 treatments when the nutrient solution amount increased from 0 to 100%. In the GN0.4 treatments, plant height was similar (about 64 cm) when nutrient solution amount was applied from 50 to 100% and was higher than that of the plants without nutrient solution (GN0.4-0, 52 cm). Again, the number plant leaves changed in a manner similar to plant height.

These results indicate that Ginate promotes hemp growth and Ginate at 0.4% can reduce the application of nutrient solution by 50%.

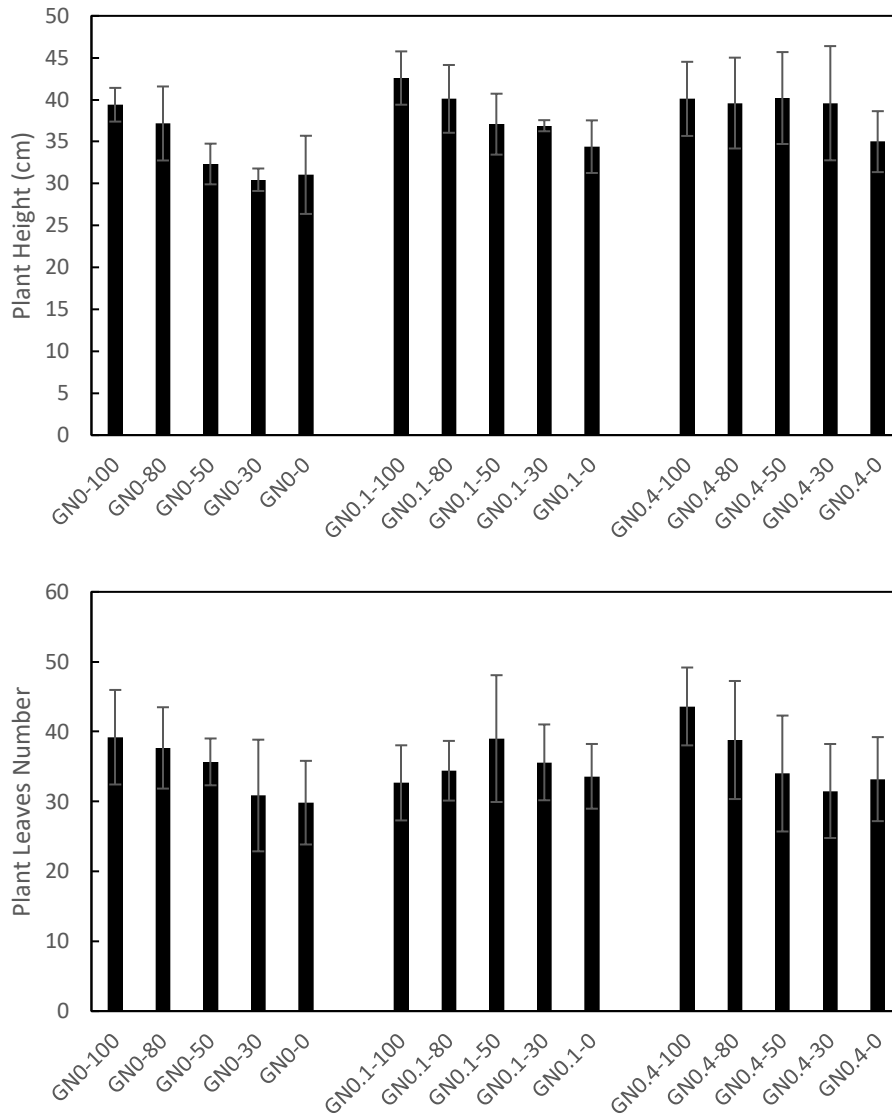


Figure 1. The plant height and leaves number of hemp plants with different treatments at 31 days. The values are the average of 5 plants of each treatment. The error bars represent the standard deviation of 5 biological replicates. GN=Ginate. GN0, GN0.1, and GN0.4 = 0, 0.1%, and 0.4% Ginate mixed in soils respectively. The different nutrient solution application amounts were indicated by 0, 30, 50, 80, and 100.

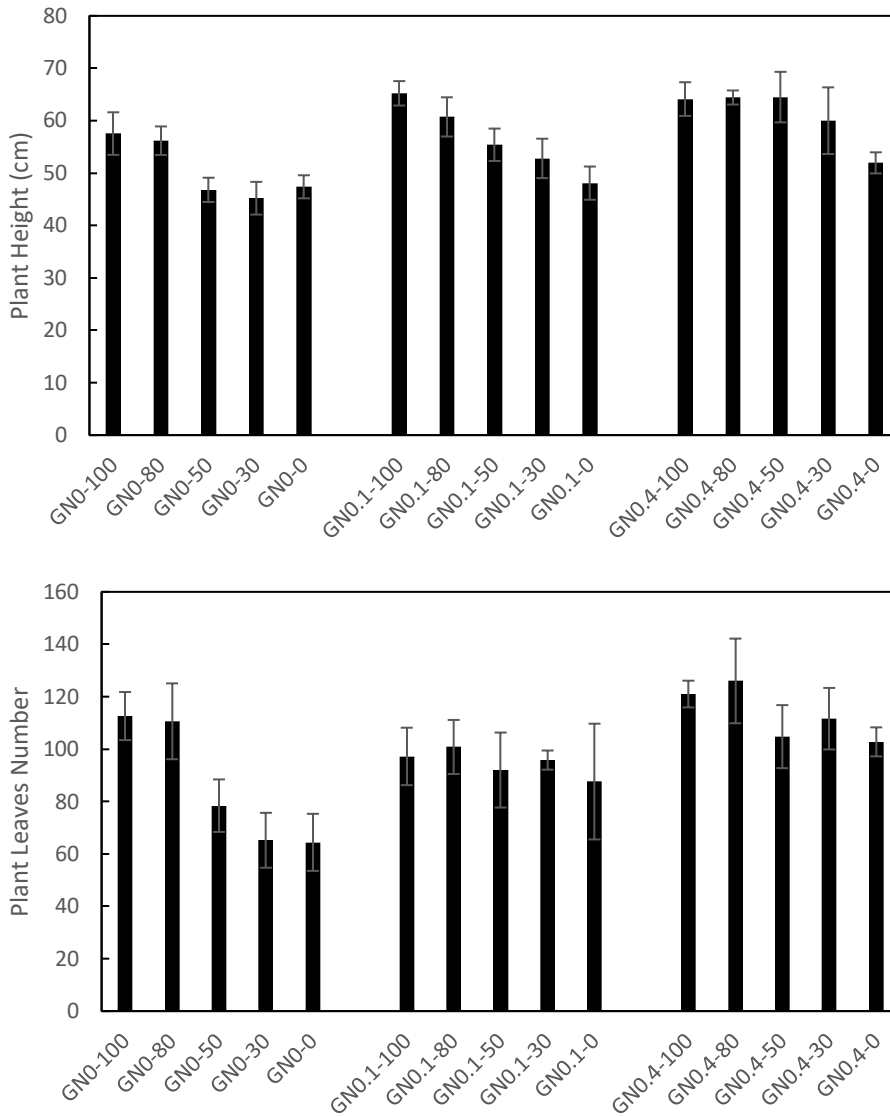


Figure 2. The plant height and leaves number of hemp plants with different treatments at 50 days. The values are the average of 5 plants of each treatment. The error bar represents the standard deviation of the 5 biological replicates. GN=Ginate. GN0, GN0.1, and GN0.4 = 0, 0.1%, and 0.4% Ginate mixed in soils respectively. The different nutrient solution application amounts were indicated by 0, 30, 50, 80, and 100.

### Effects of Ginate application on root development

#### *Purpose*

To determine the effects of Ginate on root system development.

*Experimental design*

In previous tests, it was found that 0.4% Ginate promoted growth of pepper and lettuce. In this study, the root system of plants was determined to evaluate the effects of Ginate on root parameters (root weight, length, and volume).

This study involved three plants (lettuce, pepper, and Kentucky blue grass) and three soil treatments (Ginate, commercial fertilizer, and raw coal) with unamended soil as the control/reference. Ginate was mixed in soil at 0.4% (w/w). The commercial organic fertilizer (GRO-WELL Proven Organics 4-lb Natural All Purpose Food) and an equivalent amount of raw coal (lignite) to the Ginate tests were applied in different treatments (Table 2).

Seedlings at 2-true-leaves stage were transplanted into the treatment pots. All plants were growing 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. After harvesting, the root samples were cleaned with water to remove residual soil particles and stored in a refrigerator. The root samples were scanned and analyzed with the help of WinRHIZO software (Instruments Regent Co., Ville de Québec, QC, Canada). Finally, the roots were oven dried to constant mass at 65 °C for 3 days to determine dry mass.

Table 2. Experimental design for the root study.

<b>Treatment#</b>	<b>Fertilizer Type</b>	<b>Fertilizer Amount, %</b>	<b>Plants</b>
1	None	0	Lettuce
2	Commercial Fertilizer	0.4	Lettuce
3	Coal	0.4	Lettuce
4	Ginate	0.4	Lettuce
5	None	0	Pepper
6	Commercial Fertilizer	0.4	Pepper
7	Coal	0.4	Pepper
8	Ginate	0.4	Pepper
9	None	0	Grass
10	Commercial Fertilizer	0.4	Grass
11	Coal	0.4	Grass
12	Ginate	0.4	Grass

*Results:*

As shown in Figures 3, 5, and 7, the 0.4% Ginate treatment resulted in a more developed root system for lettuce, pepper, and Kentucky blue grass plants than those of the reference and other treatments. Moreover, the fresh weight of the whole plant, root, and shoot and the dry weight of both root and shoot in the 0.4% Ginate treatment were all higher than those of the other treatments (Figures 4, 6, 8). These results indicate that Ginate promoted the growth (both below and above ground) of lettuce, pepper, and Kentucky blue grass.

In terms of the root system, it was found that the treatment of Ginate increased both the root diameter and root volume of lettuce, pepper, and Kentucky blue grass (Figures 4, 6, 8) than those of the reference and other treatments respectively which indicates the application of Ginate resulted in more developed root system of plants.

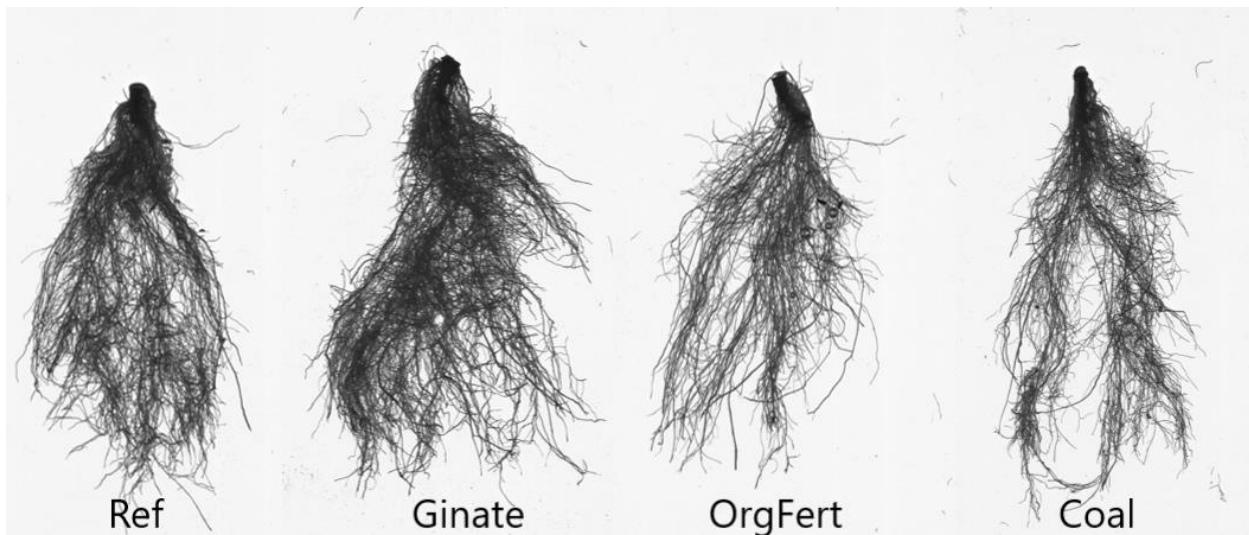


Figure 3. Images of root system of lettuce with different treatment. Ref: no addition; Ginate: 0.4% (w/w); OrgFert: 0.4% (w/w) commercial organic fertilizer; Coal: 0.4% (w/w) raw coal.

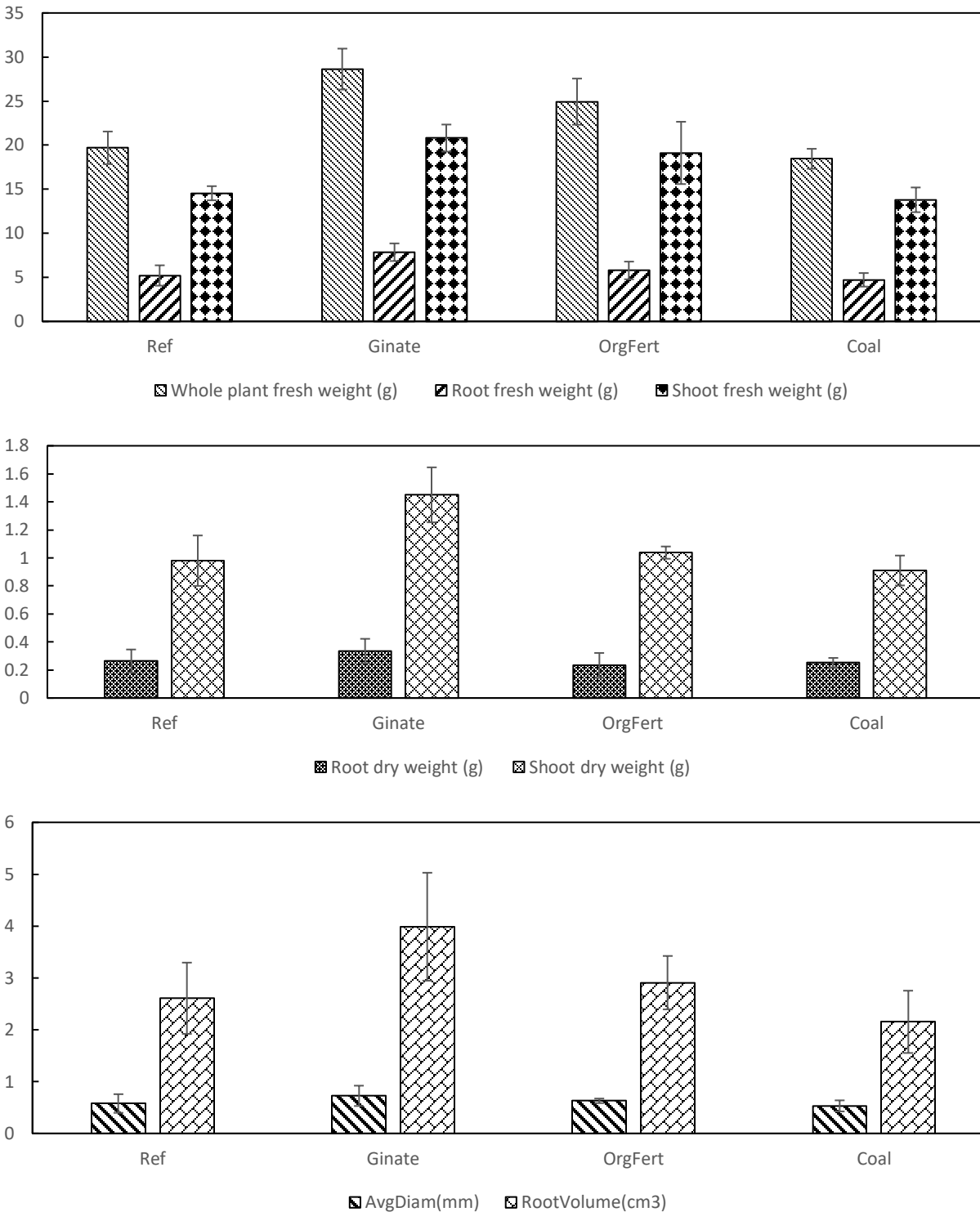


Figure 4. The fresh weight, dry weight, root average diameter, and root volume of lettuce plants with different treatments. Values are the averages from the 4 plants of each treatment. Error bars represent the standard deviation of 4 biological replicates.



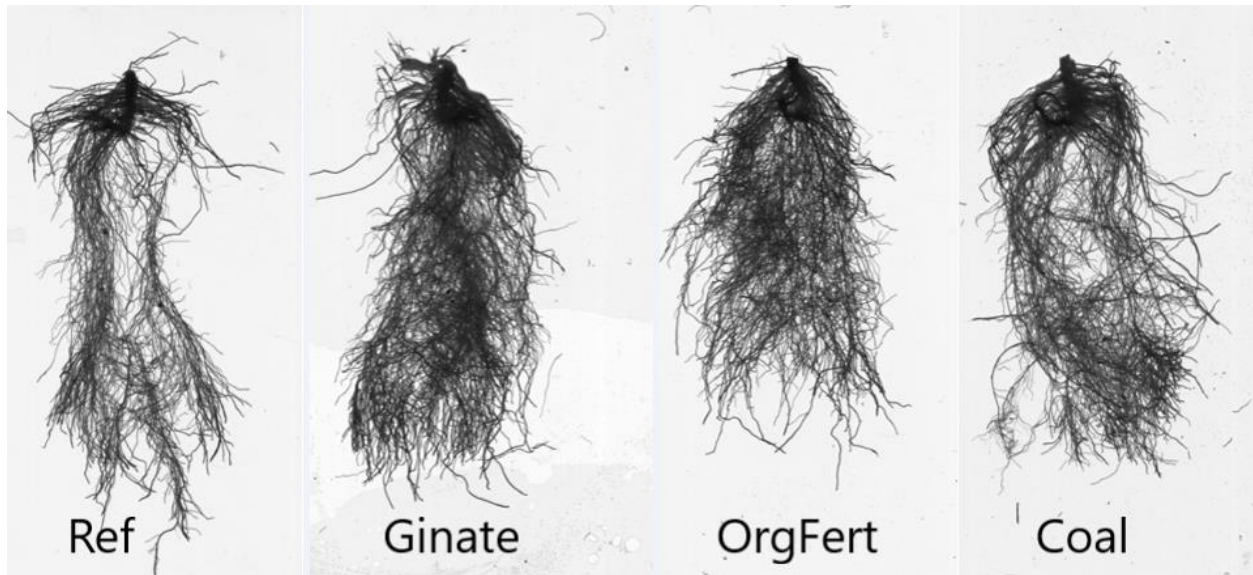


Figure 5. Images of root system of pepper with different treatment. Ref: no addition; Ginate: 0.4% (w/w); OrgFert: 0.4% (w/w) Commercial Organic Fertilizer; Coal: 0.4% (w/w) raw coal.

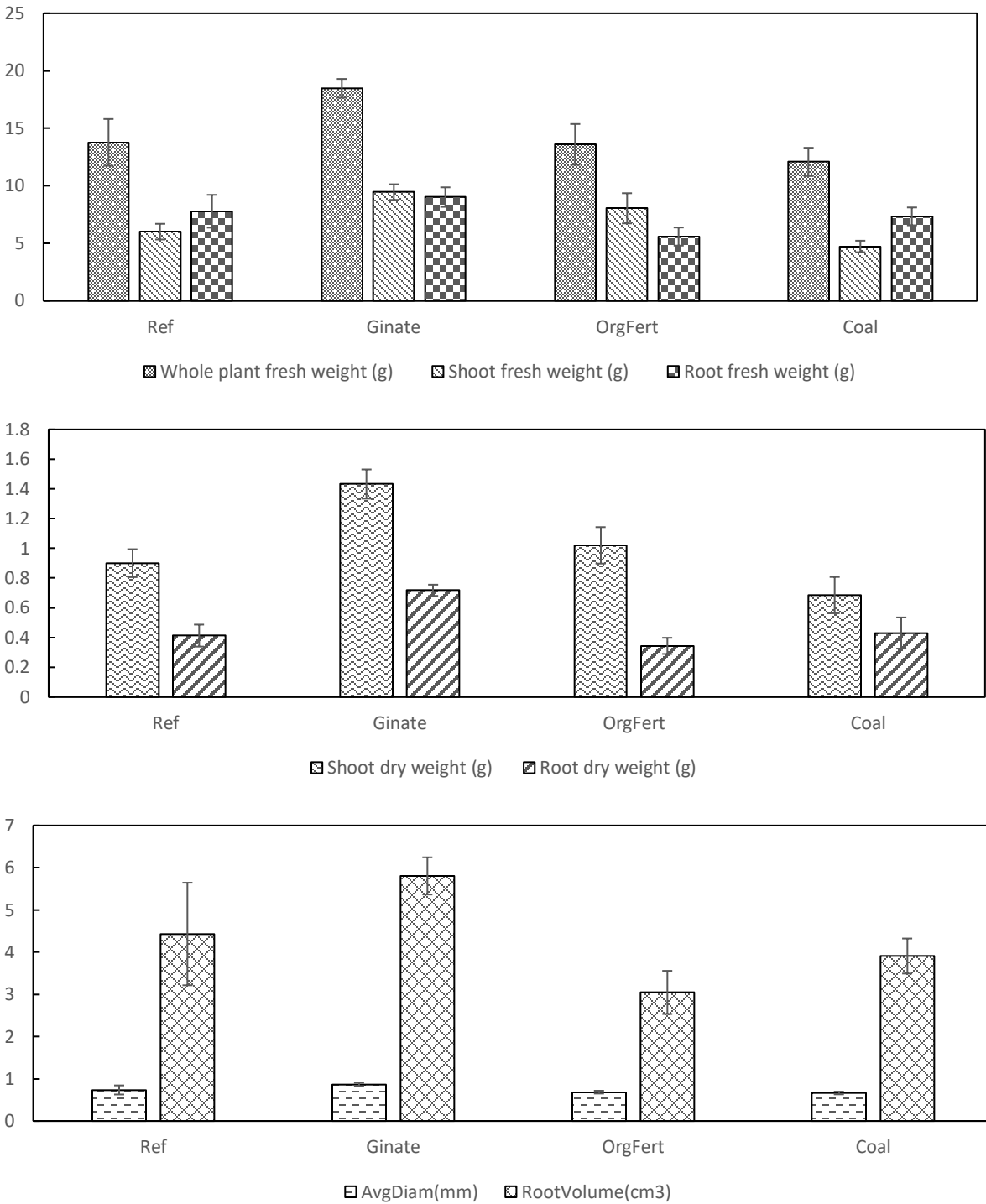


Figure 6. The fresh weight, dry weight, root average diameter, and root volume of pepper plants with different treatments. Values are the averages from the 4 plants of each treatment. The error bar represents the standard deviation of the 4 biological replicates.

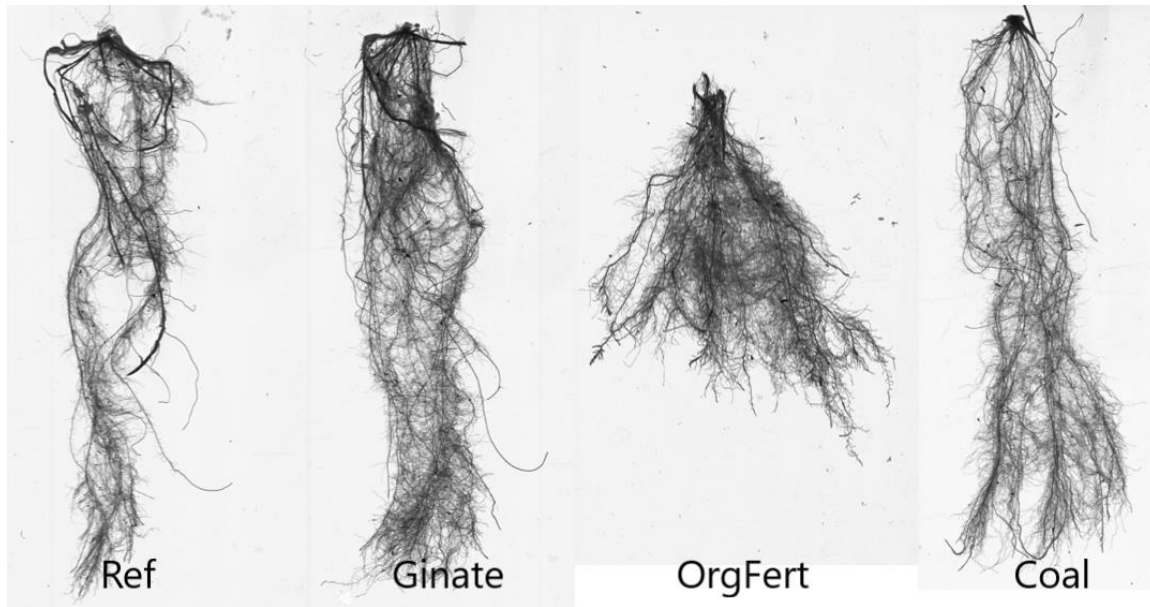


Figure 7. Images of root system of Kentucky blue grass with different treatments. Ref: no addition; Ginate: 0.4% (w/w); OrgFert: 0.4% (w/w) commercial organic fertilizer; Coal: 0.4% (w/w) raw coal.

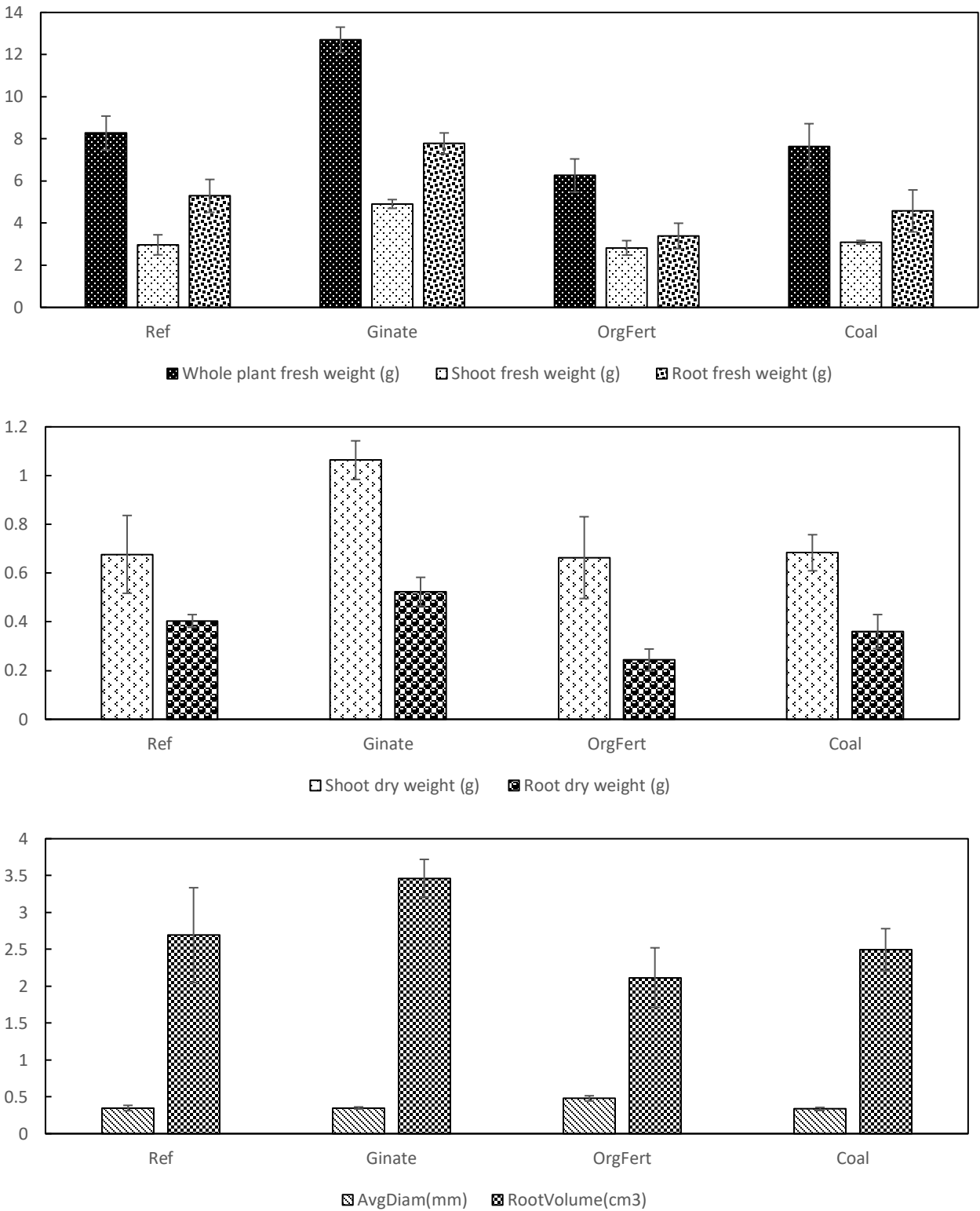


Figure 8. The fresh weight, dry weight, root average diameter, and root volume of Kentucky blue grass with different treatments. Values are the averages from the 4 plants of each treatment. Error bars represent the standard deviation of 4 biological replicates.

## Summary

We found out that Ginate promotes hemp growth and Ginate at 0.4% (w/w) can reduce the application of nutrient solution by 50%. We also found the 0.4% Ginate treatment not only resulted in higher plant biomass but also in a more developed root system in the three tested plants (lettuce, pepper, and Kentucky blue grass). Since the root system determines a plant's ability to compete for soil nutrients, the application of Ginate may improve the plant's ability to obtain more nutrients and water from soil, which would help to promote growth and productivity, and to be more tolerant to drought. However, more research is needed to validate this hypothesis and to understand the mechanism(s) behind the observed phenomena.