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Effects of Hydrogen Peroxide on Organically Fertilized Hydroponic Lettuce (*Lactuca sativa* L.)

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Abstract: Hydroponic production typically uses conventional fertilizers, but information is lacking on the use of organic hydroponic fertilizers. Development of microbial communities and biofilm that can reduce dissolved oxygen availability is a difficulty with organic hydroponics. One potential solution is the use of hydrogen peroxide (H_2O_2) which can reduce microbial populations and decompose to form oxygen. However, information is lacking on the impact of hydrogen peroxide on hydroponic crop performance. The aim of this study was to determine the effects of H_2O_2 concentrations in deep water culture hydroponics by assessing how it affects plant size and yield in lettuce (*Lactuca sativa* L.) "Rouxai". In this experiment, three H_2O_2 treatments, namely the application of 0, 37.5 or 75 mg/L H_2O_2 to 4 L aerated hydroponic containers with either conventional or organic fertilizer, were compared. The containers had either fish-based organic fertilizer (4-4-1, N-P_2O_5-K_2O) or inorganic mineral based conventional nutrient solution (21-5-20, N-P_2O_5-K_2O), both applied at 150 mg/L N. Three replicates of each H_2O_2 treatment–fertilizer combination were prepared resulting in a total of eighteen mini hydroponic containers each with one head of lettuce. There were two growth cycles: fall 2018 and spring 2019. When added to conventional fertilizers, both 37.5 mg/L and 75 mg/L of H_2O_2 led to stunted growth or death of lettuce plants. However, when 37.5 mg/L of H_2O_2 was applied to organic fertilizers, the lettuce yield nearly matched that of the conventionally fertilized control, demonstrating that the application of H_2O_2 has the potential to make organic hydroponic fertilization a more viable method in the future.

Keywords: hydroponics; organic; hydrogen peroxide; fertilizer; dissolved oxygen

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1. Introduction

Marketed as a technologically revolutionary and sustainable way to grow produce, the employment of hydroponic methods in greenhouses and "plant factories" is gaining traction globally [1]. However, chemical fertilizers typically used in hydroponics are mined from nonrenewable, finite sources or rely on fossil fuels for production, rendering them unsustainable [2,3]. Moreover, the disposal of chemically fertilized wastewater from these systems can leach into the environment and over time, degrade ecosystems as well as contaminate clean water sources [4,5].

An alternative to these conventional fertilizers is the use of organic fertilizers derived from plant and animal byproducts such as seaweed extract, manure or hydrolyzed fish emulsion [6] which require the development of microbial communities to mineralize complex organic compounds to make them plant available [7,8]. Drawbacks of organic fertilizers include variable, significantly reduced yield [9–11] which may be attributed to unstable microbial activity, difficulty supplying the proper proportion of nutrients, high pH as well as the development of biofilm in the organically fertilized hydroponic reservoirs [12]. Regarding biofilm, it is believed that the suspended organic matter which can develop on plant roots can also clog pumps/recirculation lines, reduce oxygen and nutrient uptake by roots and deplete nutrient solution oxygen levels [13–15].

There is some anecdotal evidence that the addition of hydrogen peroxide (H_2O_2) to organically fertilized reservoirs may help reduce the development of biofilm and improve the performance of organic hydroponics [16,17]. H_2O_2 is an unstable oxidizing agent most commonly used as an inexpensive, household disinfectant and bleaching agent [18]. Additionally, at low concentrations, H_2O_2 is an endogenous reactive oxygen species (ROS) which serves as an important signaling molecule in several plant functions, such as plant response to pathogens, abiotic stress, as a growth regulator and in low concentrations, can positively influence plant growth and yield [19,20].

Byproducts produced by the decomposition of H_2O_2 are H_2O and O_2. In hydroponic nutrient solutions, the released O_2 can increase the dissolved oxygen concentration in the root zone and may also help mitigate oxygen losses to biofilm and microbial respiration. Though the application of H_2O_2 is thought to help increase dissolved oxygen (DO) concentrations within the reservoir, in conventional hydroponics lettuce studies did not previously show a positive benefit of DO at or above 25% of saturation (2.1 $mg \cdot L^{-1}$) on shoot or root biomass [21]. Possible benefits of H_2O_2 in an organic hydroponic system could be increased dissolved oxygen concentration if they fall below this low threshold or alternatively may be due to H_2O_2's disinfectant properties [17].

In greenhouses, recirculated irrigation water may be chemically treated such as with ozone or H_2O_2 resulting in free oxygen radicals to control microbial growth in the recirculated water. Current research and usages of ozonated/H_2O_2 water in recirculating hydroponic systems involve continuous injection of low concentrations rather than periodic influx of higher concentrations. Furthermore, the research primarily explores its impact on microbial levels with less information on crop yield [19–24].

Currently there is little work reported in the scientific literature on the effect of periodic H_2O_2 additions on plant health and yield in conventional and organic hydroponic systems for lettuce [16], including no commonly recognized concentration or range of H_2O_2 to add to hydroponic systems. Excess H_2O_2 can also harm plant root systems in hydroponics [22,25,26], and information is lacking on the concentration that damages hydroponic crops, including lettuce. Currently suggested H_2O_2 practices vary greatly among hydroponics hobbyists and are typically determined on a trial and error basis. With little to no scientifically backed information available on the topic, this study aimed to explore the usage of H_2O_2 on dissolved oxygen in the root zone and its effects on yield in conventionally and organically fertilized lettuce heads.

2. Materials and Methods

2.1. Experimental Seedling Preparation

The experiments were conducted at the Cornell University Kenneth Post Laboratory greenhouses in Ithaca, New York with average daily temperature controlled to 22 °C and with ambient lighting. The first crop cycle took place from November through December 2018 and the second crop cycle took place March to April 2019. Light intensity was not logged directly in the greenhouse; however, the greenhouse had an outdoor weather station logging photosynthetic photon flux density (PPFD, $\mu mol \cdot m^{-2} \cdot s^{-1}$) which was used to calculate outdoor daily light integral (DLI, $mol \cdot m^{-2} \cdot d^{-1}$). Outdoor DLI averaged 6.93 and 17.31 $mol \cdot m^{-2} \cdot d^{-1}$ during the fall 2018 and spring 2019 experimental periods, respectively. Greenhouse light transmissivity was estimated to be 50%. Therefore, estimated greenhouse DLI averaged 3.47 and 8.65 during the fall 2018 and spring 2019 experimental periods, respectively.

A flat with 1.5 × 1.5 inch rockwool plugs was soaked in reverse osmosis water for approximately 10 min and was then drained and placed on a plastic flat. Each rockwool cell was seeded with one pelleted (*Lactuca sativa* L.) "Rouxai" seed (Johnny's Seeds, Fairfield, ME) and was allowed to germinate in a seedling production area in the greenhouse under 18 h lighting from high pressure sodium (HPS) lamps. The seedlings were watered daily with fertilized water (Jack's Professional LX 21-5-20 All Purpose Water-Soluble Fertilizer supplemented with magnesium sulfate so as to supply 30 ppm mg). Seedlings were transplanted into 4 L hydroponic containers after 21 days.

2.2. Treatment Setup

After 21 days when the seedlings had 3 to 4 true leaves, 18 individual 4 L buckets were prepared for the experiment. Each bucket was filled near to capacity with reverse osmosis water. The conventional fertilizer, Jack's Professional LX 21-5-20 All Purpose Water-Soluble Fertilizer (JR Peters Inc., Allentown, PA, USA) with magnesium sulfate was applied to half of the buckets. The other half of the buckets were fertilized with the organic fertilizer, Drammatic One 4-4-1 Fish Emulsion (Dramm Corporation, Manitowoc, WI) and in both cases the fertilizers were added to supply an electrical conductivity (EC) of 1.5–1.7 dS/m. For the 21-5-20 conventional fertilizer treatment the mineral nutrient concentration was 150 mg·L^{-1} nitrogen (N), 15.6 mg·L^{-1} phosphorus (P), 118.6 mg·L^{-1} potassium (K), 30 mg·L^{-1} magnesium (Mg), 40.2 mg·L^{-1} sulfur (S), 0.75 mg·L^{-1} iron (Fe), 0.38 mg·L^{-1} manganese (Mn), 0.38 mg·L^{-1} zinc (Zn), 0.19 mg·L^{-1} boron (B), 0.19 mg·L^{-1} copper (Cu) and 0.075 mg·L-1 molybdenum (Mo). For the Drammatic One organic fertilizer the mineral nutrient concentration was 150 mg·L^{-1} N, 16.4 mg·L^{-1} P and 15.6 mg·L^{-1} K (the label did not list secondary macronutrients or micronutrients). Household 3% H_2O_2 was added to the buckets according to the treatments in Table 1 (additions of 0, 1.25 or 2.5 mL/L of 3% H_2O_2 resulting in 0, 37.5 and 75 mg·L^{-1} H_2O_2) with 3 replications of each treatment combination.

Table 1. Names and descriptions of experimental treatments.

Treatment	Description
CONV_0	Conventionally fertilized control
CONV_37.5	Conventionally fertilized with 37.5 mg/L H_2O_2
CONV_75	Conventionally fertilized with 75 mg/L H_2O_2
ORG_0	Organically fertilized control
ORG_37.5	Organically fertilized with 37.5 mg/L H_2O_2
ORG_75	Organically fertilized with 75 mg/L H_2O_2

The buckets were arranged on a bench in the greenhouse in 3 rows of 6 buckets spaced approximately 30 cm apart. Each bucket was individually aerated with an airstone placed near the rootzone that was powered by air pumps (GH2716, General Hydroponics, Santa Rosa, CA, USA) as shown in Figure 1 and were randomly arranged by treatment.

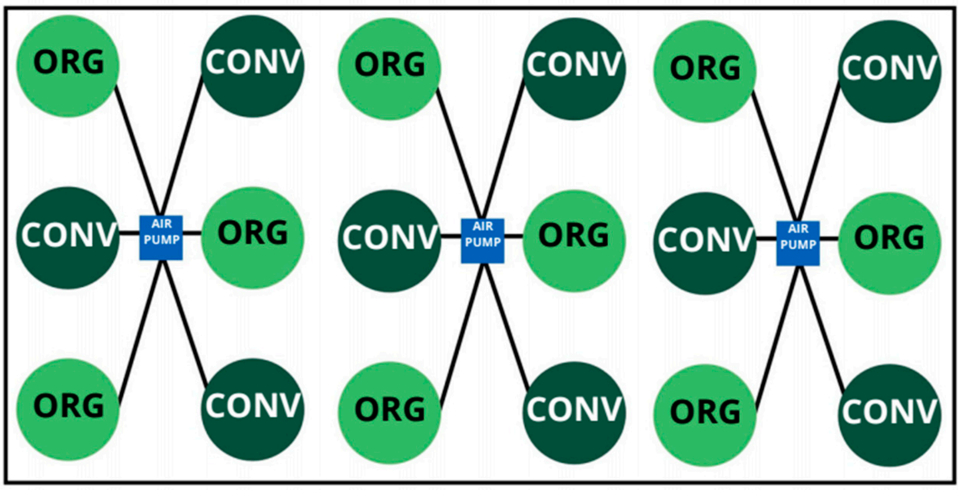


Figure 1. Illustration of experimental set up.

The electrical conductivity (EC) of each bucket was measured using an EcoTestr CTS meter (Oakton, Vernon Hills, IL, USA) and was adjusted to 1.5–1.7 dS/m. Additionally, the pH levels were measured using an EcoTestr pH2+ meter (Oakton, Vernon Hills) and were adjusted to 5.5–6 with either nitric acid (1 M HNO_3) to lower the pH or potassium hydroxide (1 M KOH) to bring pH levels up.

Eighteen uniform lettuce seedlings were selected from the seedling production area and each plant was placed in the 1″ diameter hole drilled into the center of each lid so that the rockwool plugs fit snugly. The buckets were checked to make sure the water levels were high enough to reach the plants' roots. Dissolved oxygen (DO) measurements were taken using a YSI Pro20 Dissolved Oxygen Meter (Xylem Inc., Yellow Springs, OH, USA) and results were expressed on a percent of DO saturation at the recorded water temperature.

2.3. Experimental Design, Data Collection and Statistical Analysis

Each day, EC and pH were adjusted and maintained between the target values (1.5–1.7 dS/m and pH 5.5–6) using the respective tools, and reservoir water levels were topped-off daily to maintain 4 L of nutrient solution. DO measurements were recorded daily using the YSI Pro20 Dissolved Oxygen Meter.

Every 3 days, hydrogen peroxide was added according to the treatment prior to the DO measurements for that day. During the fall trial, this was done for 17 days and on the 18th day measurements were taken of the following: root length from the bottom of the rockwool plug to the root tip, average leaf width and plant height from the top of the rockwool plug measured with a ruler. Then, lettuce was harvested by severing the plant where the plant stalk met the rockwool plugs and final fresh weight was recorded. In the spring trial, the fall cycle was replicated except a longer crop cycle of 26 days was conducted and on the 27th day, data was collected on root length and fresh weight but due to time constraints, DO concentration, leaf width and plant height data was not collected. At the time of harvest, visual observation showed no biofilm development in the conventional treatments and some in the organic treatments.

The experiment was designed as a completely randomized design (CRD) with 6 treatment combinations (2 fertilizers $\times$ 3 H_2O_2 concentrations). There were 3 replicate experimental units (represented by a hydroponic bucket with 1 plant) for each treatment combination. The experiment was performed twice (fall and spring). The data were analyzed using JMP software (SAS Institute, Cary, NC, USA). ANOVA was used to determine if there was a significant effect of crop trial (fall vs. spring) but no trial by treatment interaction. Tukey's Honestly Significant Difference (HSD) Test (α = 0.05) was used to determine differences among treatments for each trial.

3. Results

3.1. Dissolved Oxygen

In the fall trial, dissolved oxygen (DO) levels were recorded each day to track the degradation of H_2O_2 within the rootzone (Figure 2). DO was added to the hydroponic containers three times weekly (represented by days 0, 3, 6, 9, 12, 15 . . .). Steep increases in DO levels represented days in which H_2O_2 was added to the reservoirs. It was noted that on average, organically fertilized treatments saw more drastic swings in DO levels than their conventional counterparts and that over time, the application of H_2O_2 had less effect on DO levels. Conventional fertilizer with 75 mg/L H_2O_2 led to the greatest sustained levels of DO, and in the organic treatments DO levels degraded more quickly after each addition.

3.2. Fresh Weight

The plants in the CONV_0 treatment had the highest mean value, with no statistically significant difference between this treatment and organic or conventional treatments with 37.5 mg/L H_2O_2 (Figure 3). The conventionally fertilized control yield was nearly double that of the organically fertilized with 0 mg/L H_2O_2. With the lower application of H_2O_2 however, ORG_37.5 had a statistically similar fresh weight (FW) to CONV_37.5 and CONV_0. At the greater application of 75 mg/L H_2O_2, both ORG_75 and CONV_75 yield decreased with the CONV_75 plants dying and therefore having a lower FW than all other treatments. Across treatments, this shows that application of H_2O_2 had a negative impact on conventional treatments but the low dose (37.5 mg/L) actually increased yield of organic treatments to the point that it was not significantly different from conventional fertilizer.

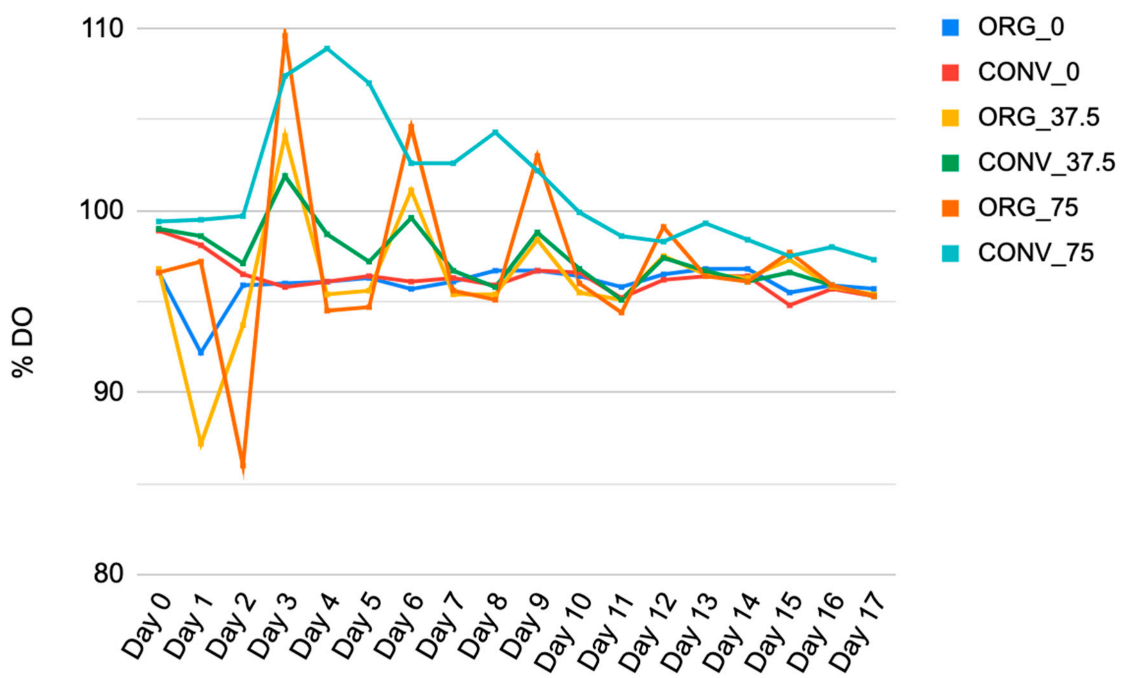
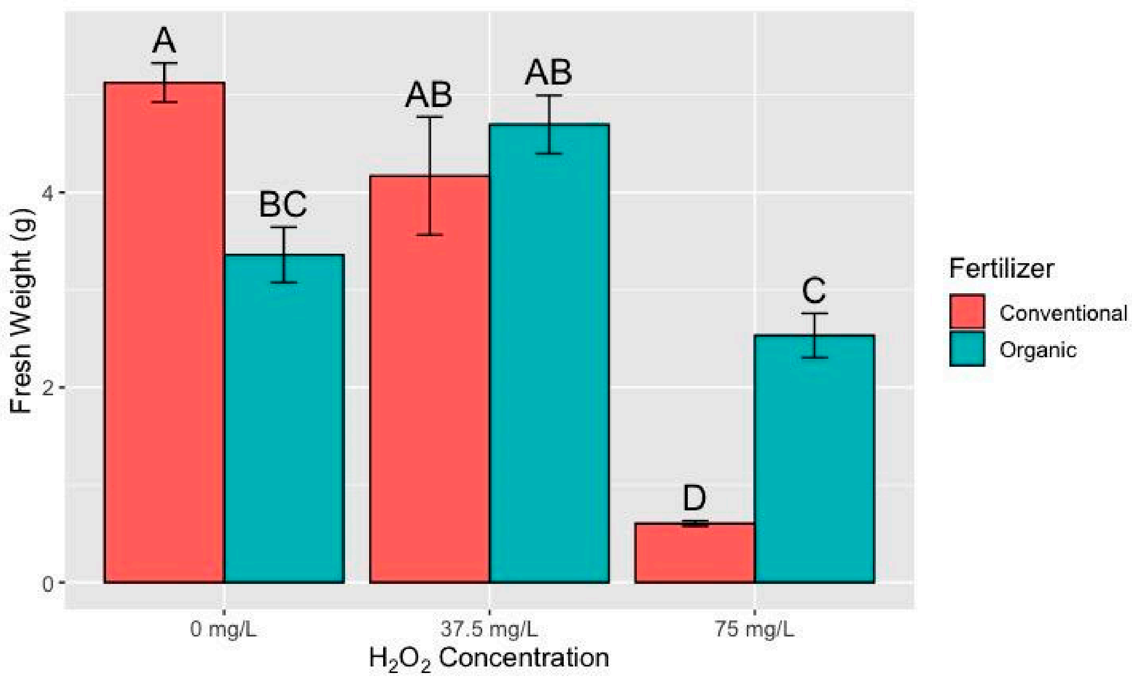
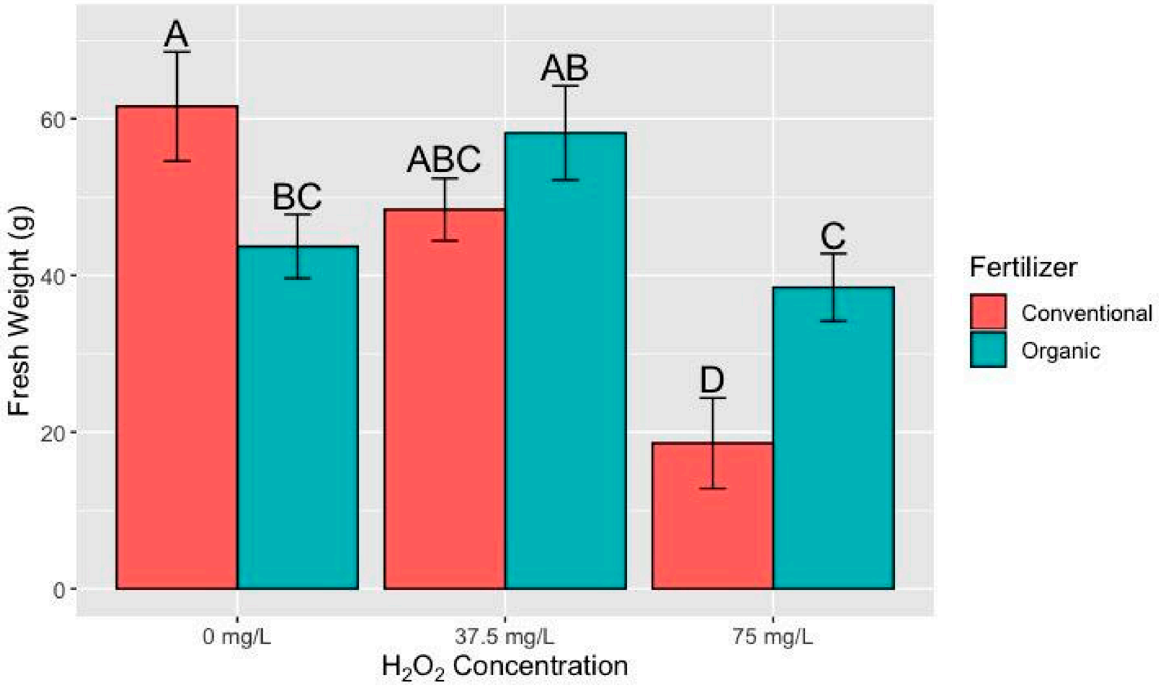


Figure 2. Percent dissolved oxygen levels taken within lettuce plant root zones in the fertilizer and H_2O_2 treatments in the Fall 2018 trial. Data are means of 3 plants per treatment combination per day.



(**a**)

Figure 3. *Cont.*



(**b**)

Figure 3. Fresh weight of lettuce plants in response to organic and conventional fertilizer and addition of H_2O_2 in the fall (**a**) and spring (**b**) trials. Data are means ± SE of 3 plants per treatment combination. Letters represent mean separation using Tukey's HSD (α = 0.05).

During the spring 2019 trial, differences between treatments remained similar confirming the results from the fall 2018 trial (Figure 3). However, overall fresh weight was greater in the spring trial likely due to greater ambient light and the longer crop cycle compared to the fall trial. Additionally, though their growth was severely stunted, the plants grown with the CONV_75 and ORG_75 treatments with high doses of H_2O_2 did not die.

3.3. Root Length

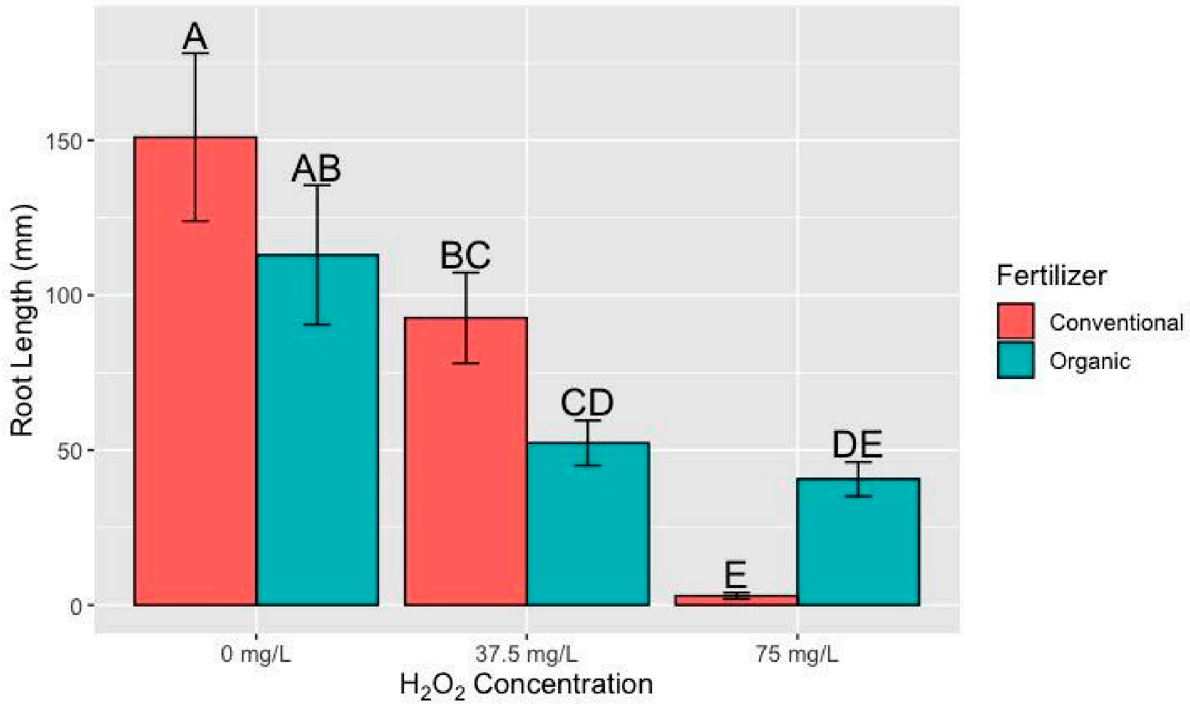
In the fall trial, plants in the CONV_0 and ORG_0 treatment had the highest mean root length (Figure 4). The application of H_2O_2 dramatically decreased root length for both fertilizer treatments but the effects were more dramatic for conventionally fertilized treatments.

In the spring trial, similar patterns were found. There was no difference in root length between the conventional and organic controls (0 ppm H_2O_2), but as H_2O_2 treatments increased, root length dramatically decreased, especially in the conventionally fertilized treatments.

3.4. Leaf Width and Plant Height

For the fall trial, data was collected on leaf width and plant height. Due to time constraints these parameters were not collected in the spring trial. For leaf width, the only statistically significant difference was that CONV_75 had a smaller leaf width (about half the size) to all other treatments (Figure 5).

Likewise, for plant height, similar effects were found where the height of conventionally fertilized plants at 75 mg/L H_2O_2 was dramatically less than other treatments. For organic fertilization, the plants at 75 mg/L H_2O_2 were shorter than conventional plants at 37.5 mg/L H_2O_2 (Figure 6).



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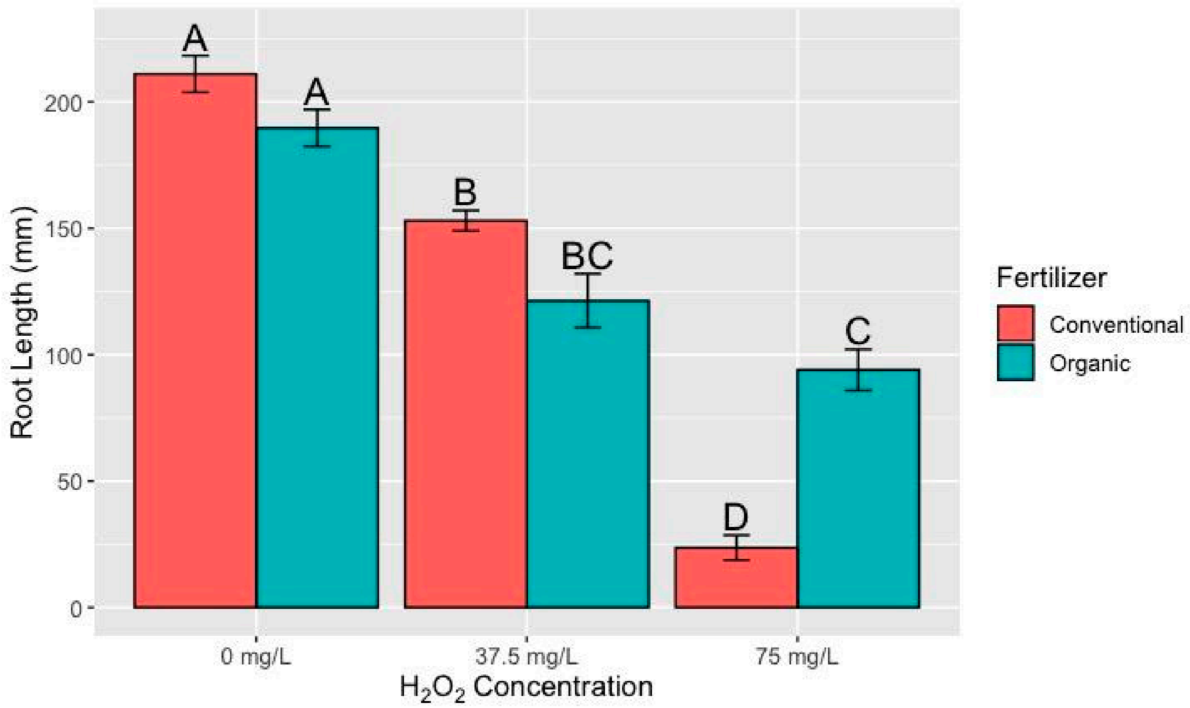

(**b**)

Figure 4. Root length of lettuce plants in response to organic and conventional fertilizer and addition of hydrogen peroxide in the fall (**a**) and spring (**b**) trial. Data are means ± SE of 3 plants per treatment combination. Letters represent mean separation using Tukey's HSD (α = 0.05).

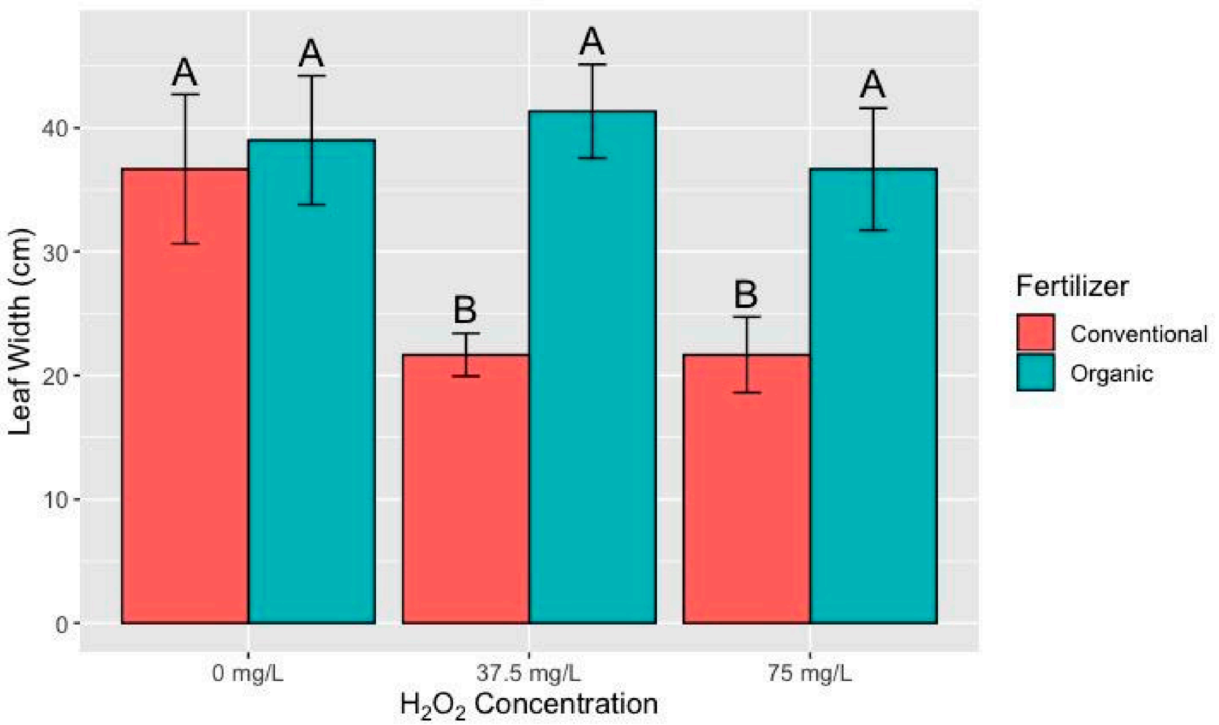

Figure 5. Leaf width of lettuce plants in response to organic and conventional fertilizer and addition of hydrogen peroxide in the fall trial. Data are means ± SE of 3 plants per treatment combination. Letters represent mean separation using Tukey's HSD (α = 0.05).

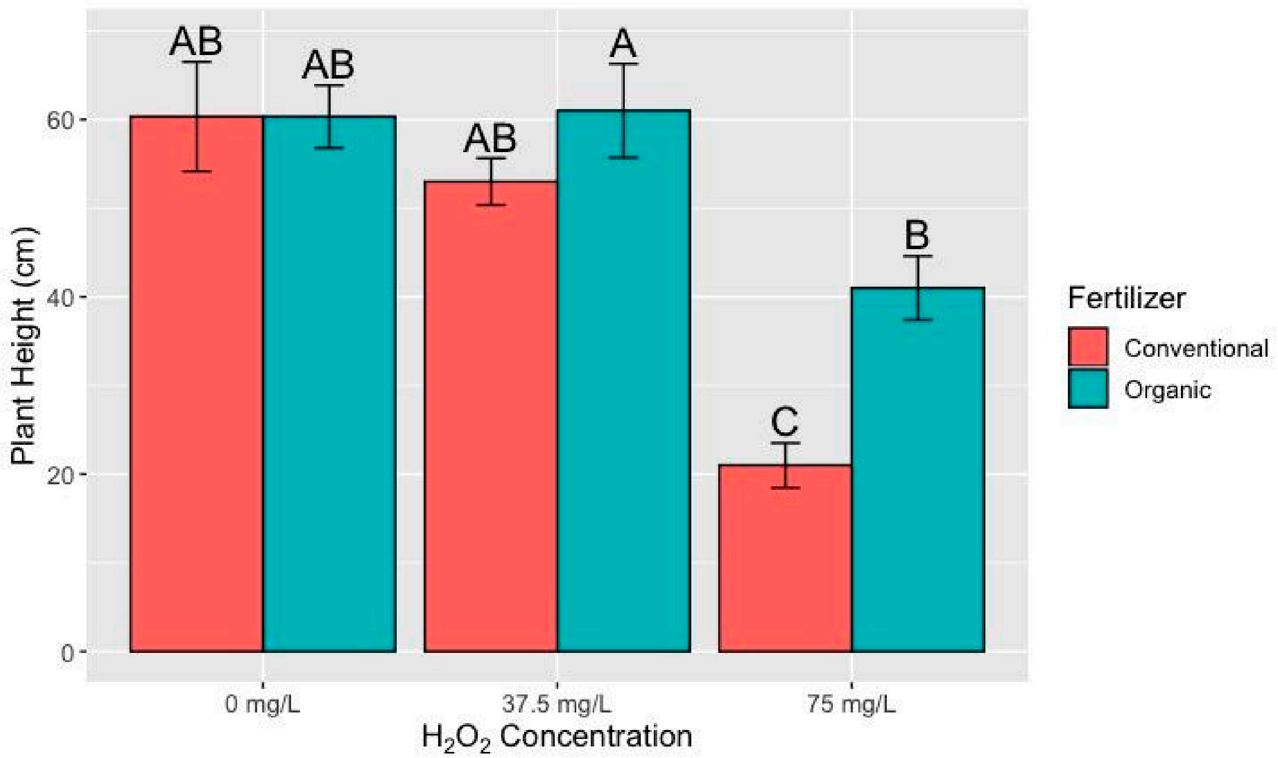

Figure 6. Plant height of lettuce plants in response to organic and conventional fertilizer and addition of hydrogen peroxide in the fall trial. Data are means ± SE of 3 plants per treatment combination. Letters represent mean separation using Tukey's HSD (α = 0.05).

4. Discussion

The results of this study show the potential for the integration of H_2O_2 with organic fertilizers to optimize hydroponic lettuce yield. Without H_2O_2, our study found that organic fertilization performed poorer (fresh weight, root length) than conventional fertilizer (Figure 3a,b and Figure 4a,b). Our findings with organic fertilization performance (without H_2O_2) are similar to those reported by Atkin and Nichols [11]. For example, Atkin and Nichols also tested hydrolyzed fish emulsion-based fertilizer and found that organic hydroponic lettuce had approximately 55% lower fresh weight than conventional hydroponic lettuce. Results from the conventional and organic controls matched the percent yield ranges of other studies as well [11,27,28]. H_2O_2 additions reduced the performance of conventionally fertilized plants at all levels and organically fertilized plants at the higher level of treatment (75 mg/L). In fact, the application of 75 mg/L of H_2O_2 for both conventional and organic treatments led to early plant death. At the lower treatment of H_2O_2, the conventionally fertilized plants had somewhat stunted growth (Figure 3a,b). Most interestingly, at moderate H_2O_2 concentration (37.5 mg/L) the organic fertilizer plants performed as well as control plants (0 mg/L H_2O_2) with conventional fertilizer.

H_2O_2 functions by decomposing into an unstable free radical oxygen molecule which can destroy biotic cell tissue. As such, H_2O_2 has the potential to indiscriminately damage healthy living root tissue, consequently reducing the fresh weight of lettuce heads in higher doses. Root damage may be the result of this phytotoxicity [17,29–31]. However, when H_2O_2 at 37.5 mg/L was added to organic fertilizer, this treatment performed as well as conventional fertilizer without H_2O_2. We hypothesize that the lack of negative impacts of 37.5 mg/L H_2O_2 in the organic fertilizer treatment were due to the effect of biofilm present in the rootzone; the free radical oxygen molecules may have disrupted biofilm matter thereby leading to less damage to roots. Since the conventionally fertilized reservoirs did not contain visible biofilm, this may have led to higher levels of root damage (Figure 4a,b), and subsequently, lower lettuce fresh weight. Additionally, as an ROS, the addition of H_2O_2 may have positively impacted plant growth through improving plant responses to stress, though the impact of H_2O_2 application at the concentration used in this study likely outweighed the benefits in most treatments [16].

Further, while the effects of the application of H_2O_2 had a visible impact on the plant material, there were no visible reductions in biofilm development among the organic treatments. This suggests that, while it may be effective in increasing yield, manual disinfection of hydroponic systems would still be needed in between growth cycles to clear out the biofilm that may clog and stick to surfaces. Thus, future research would be needed to quantify the impact of H_2O_2 on biofilm in hydroponics, as well as to investigate the extent of root tissue damage as a result of H_2O_2 and if the addition of H_2O_2 has similar effects on microbial composition and nutrient balance as ozonated water.

5. Conclusions

At 0 mg/L H_2O_2, the organic fertilizer performed poorer than the conventional fertilizer, consistent with existing literature. While both 37.5 and 75 mg/L H_2O_2 added every three days led to reduced performance of conventionally fertilized plants, 37.5 mg/L H_2O_2 led to greater performance of plants receiving organic fertilizer treatments. More research is needed to determine if the response is due to (1) the increase in dissolved oxygen content of the root-zone as H_2O_2 disassociated, (2) the effects of H_2O_2 on biofilm development (i.e., injuring biofilm but allowing for greater nutrient or dissolved oxygen access by roots) or (3) the effect of Fenton reactions between H_2O_2 and ferrous iron naturally present in organic fertilizer sources on the chemical makeup (nutrient availability) of the organic hydroponic nutrient solution [32].

Future research should seek to understand the optimum concentration and reapplication rate of H_2O_2 in organic hydroponic fertilization (including lower concentrations) as well as to understand the mechanism for the response so that we have a greater understanding of effective organic hydroponic fertilization strategies.

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