Growth, yield, and quality in hydroponic vertical farming – Effects of Phycocyanin-rich Spirulina Extract

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ABSTRACT

Vertical farming (VF) is a source of high-quality, accessible, and climate-friendly nutrition for rapidly growing urban populations. To realize VF's potential as a food source, innovative technologies are required to ensure that VF can be industrialized on a massive scale, and extended beyond leafy greens and fruits into the production of food staples or row crops. A major obstacle to the economic sustainability of VF is the amount of lighting energy consumed and it is important to consider the implications of this energy consumption in terms of both environmental impact and profitability. While technological advances have improved the energy efficiency of VF lighting systems, there has been insufficient research into biological approaches to save energy by shortening the growth period from seeding to harvest. We conducted a controlled trial to evaluate the biostimulant (non-nutrient, biological growth improvement) potential of a phycocyanin-rich Spirulina extract (PRSE) in hydroponically grown, vertically farmed lettuce. PRSE application reduced the time from seeding to harvest by six days, increased yield by 12.5% and resulted in improved quality including color, taste, texture, nutritional flavonoid levels, and shelf life. This preliminary study demonstrates that natural biostimulants such as PRSE may play an important role in improving the economic sustainability of the nascent, but rapidly growing VF sector.

Keywords: *vertical farming, controlled environment agriculture, plant factories, biostimulant, microbiome, hydroponics, aeroponics*

INTRODUCTION

Climate change, food security challenges, and environmental degradation due to large-scale outdoor industrial farming make it vital to explore moving food production closer to rapidly urbanizing populations. As a result, there has been a surge of public, government and investor interest in controlled environment, multi-layer plant production, generally known as vertical farming (VF) [Kozai et al. 2020]. Vertical farms can be operated using high levels of automation including phenotype-driven, AI (artificial intelligence)-based controls to fully optimize production inputs, including lighting, environmental conditions, and nutrient delivery. VF has rapidly transitioned from a promising food production concept into an accepted technology for providing fresh leafy greens to our cities [Frazier 2017]. Leading VF companies have raised hundreds of millions of dollars and a growing consensus is emerging that VF offers a promising food production option that could not only reduce the need for valuable farming land but also decrease the use of chemical pesticides. However, the economic viability of VF remains debatable due to high capital expenditure and energy input costs, and it is still unclear as to whether VF is indeed a truly economically and environmentally sustainable solution as the prime source of leafy greens for large cities [Goodmann and Minner 2018].

To date, VF optimization research has focused overwhelmingly on photobiology (improving plant physiological parameters with lighting technology), photomorphogenisis (light-induced plant development), and photosynthesis [Sharathkumar et al. 2020]. Current growing systems using aeroponic and hydroponic technologies ensure optimal nutrient access and careful cultivar selection, both of which increase the likelihood of reaching close to the best possible production levels in the presence of fully optimized lighting conditions. Much of the focus on increasing the efficiency of VF relates to incremental improvements in lighting to ensure lower energy consumption and maximum appropriate plant light exposure [Nicole et al. 2016]. Further challenges in VF include post-harvest quality (shelf-life, color, flavor, and organoleptic properties) and nutritional density (richness in carotenoids, flavonoids, anthocyanins, and antioxidants). While it may be possible to increase levels of micronutrients in vertically farmed crops, this work is still at an early stage [Sambo et al. 2019]. Research into methods of increasing the concentration of antioxidants such as flavonoids in row crops, thereby improving nutritional quality, may be applicable in VF [Kim 2021] [Moreno Escamilla et al. 2020].

VF uses a limited number of highly-purified, bacteriologically-inert production inputs in order to avoid hydroponic and aeroponic farming problems including nozzle blockages and microbial contamination. The focus on creating low-microbial density growing conditions has largely precluded research and development into inputs that are widely used in conventional agriculture, such as live microbial amendments, humic acid, and seaweed extracts. Algal extracts used as biofertilizers (organic compounds from living microorganisms that promote plant growth) and biostimulants (formulated biological products for improving plant growth that are not nutrients, growth regulators, or plant protection agents) increase growth velocity, yield, plant vigor and disease resistance in leafy green and row crop outdoor agriculture. Algae extracts and biostimulants also have considerable utility in regenerative and organic agriculture [Yakhin et al. 2017][Win et al. 2018][Chanda et al. 2020]. Biofertilizers and biostimulants offer safer alternatives to the chemical fertilizers used in large-scale farming [Bhardway et al. 2014]. Algae extracts have demonstrated biostimulant properties, improving germination, growth, photosynthetic activity, and yield and acting at the level of the phyllosphere and rhizosphere microbiome [Chiasese et al. 2018]. Algal extracts have been demonstrated to upregulate genetic pathways associated with increased growth and yield in hydroponic sugar beet and tomato plant cultivation [Barone et al. 2018a][Barone et al. 2018b]. Spirulina (*Arthrospira platensis*), a blue-green microalgae, is a source of micronutrients and phytohormones (gibberellins, auxins, cytokinins) and Spirulina filtrates and homogenates have been shown to improve growth and nutritional quality in radish plants [Osman et al. 2016][Godlewska et al. 2019].

There has been little research into the potential agricultural applications of phycocyanin, a water-soluble pigment-protein complex phycobiliprotein, derived from Spirulina. Phycocyanin is generally used as an FDA-approved blue food colorant and food supplement. Because less than 15% phycocyanin by weight may be extracted from Spirulina, prices remain elevated and supply is generally limited. We conducted a scoping, controlled trial to explore the utility of a phycocyanin-rich Spirulina extract (PRSE) as a biostimulant in VF. While the focus of this work was to explore and quantify the impact of phycocyanin on plant growth velocity and yield as a potential approach to improve the economic sustainability of VF, we also examined the effect of PRSE on photosynthetic efficiency and quality (nutritional value, organoleptic properties and shelf life).

Methods

Phycocyanin-rich Spirulina Extract (PRSE) characterization

PRSE was produced using an aqueous, solvent-free extraction method [Lerer 2018]. The protein structure of PRSE, C-phycocyanin reference (Sigma-Aldrich, St. Louis, MO, USA) and commercially available Spirulina powder (Nutreco, Hawaii, USA) were compared using the following method:

Pulverized extracts were homogenized using a TissueLyser II (Qiagen, Hilden, Germany) in Tissue Lysis Buffer (BioRad Laboratories, Hercules, CA, USA) prior to acetone precipitation. The protein precipitates were resuspended in 0.5 M triethylammonium bicarbonate, 1 M urea and 0.1% sodium dodecyl sulfate (Sigma-Aldrich, St. Louis, MO, USA) and aliquots of the precipitated proteins were collected for LabChip analysis (LabChip GX II, Caliper Life Sciences, Waltham, MA, United States).

Plant materials, hydroponic growth conditions, and treatments

The treatment and control lettuce plant models selected were Salanova® Red Sweet Crisp for growth and phenotype studies and Latuca Sativa (Kuting, USA) for the quality studies. The plants were grown indoors in a monitored, controlled environment using a dynamic, 100 liter tray-reservoir, shallow water culture hydroponic system that held approximately 50 plants per system. Two independent systems, filled with deionized water, were constructed to ensure similar light exposure and environmental conditions for both the treatment and control plants. Plants were germinated and grown on rockwool gro-blocks (Grodan, USA), cultivated at 25° C, and exposed to a photoperiod of 16 hours daylight at 18000 Lux. All plants were nourished with a standard hydroponic 8-15-36 NPK nutrient solution (General Hydroponics Floragro, USA) at 600-700 ppm with regular adjustment to a pH of 6. The treatment group water was dosed with sufficient PRSE to ensure a media phycocyanin concentration of 125mg/L, with weekly concentration measurements [Bennet and Bogorad 1973].

Growth, phenotype, yield, colorometric and photosynthesis measurement

The growth period was assessed as the time from planting to harvest. Harvesting was undertaken when two blinded, experienced hydroponic growers reached consensus that the plants were at the most optimal stage of growth and marketable. Leaf length and basal stem width were measured at the end of the trial period using graduated calipers and total biomass (lettuce suitable for packaging) was measured at the end of the trial period. Fluorescence emission measurements were performed just prior to harvest on selected leaves of 10 randomly selected plants per group using a FluorPen FP100max fluorometer (Photon System Instruments, Brno, Czech Republic). Maximum Quantum Yield (the ability of Photosystem II to transform light into electrodes in the photosynthetic chain) and Performance Index (the overall balance of light absorption, quantum yield of photosynthesis and electron transfer in the photosynthetic chain) were measured. Visible color was assessed using a Nix™ Pro color sensor (Nix Sensor Ltd., Hamilton, Ontario, Canada). 10 plants were randomly selected from both control and treatment groups and 10 leaves were randomly selected from each group at the conclusion of the trial to assess CIELAB (color expressed as three values: L^* for the lightness from black (0) to white (100), a^* from green (−) to red (+), and b^* from blue (−) to yellow (+)) [Post 2020].

Quality measurement

Six random samples were sourced from both control and treatment groups and subsequently packed in supermarket-style, transparent clamshells and stored at 16°C at 70% humidity in a controlled environment chamber. Daily, blinded assessment was undertaken for wilting and color loss. Flavonoids analysis was performed using LC-MS [Seal 2015; Wang et al. 2014]. A Flexar HPLC system (Perkin Elmer, Waltham, MA, USA) and an expression CMS (Advion, Ithaca, NY, USA) were used and separation was done on a Brownlee SPP C18 column (2.7μm x 150mm x 3.0mm) (Perkin Elmer, Waltham, MA, USA) with a mobile phase flow rate of 0.2 mL/min. The first mobile phase was 10% methanol, 85.5% water, and 4.5% formic acid (v/v) and the second, was 80% methanol, 19% water, and 1% formic acid (v/v). Sample injection volume was 20 μL, and the separation was run at 25°C. The flavonoid standards were Quercetin and Luteolin (Sigma-Aldrich, St. Louis, MO, USA). Blinded organoleptic testing (by an independent standards laboratory) was conducted on 6 harvested samples from each group assessing aroma, taste and texture [Csajbokne 2018].

Statistical analysis

The treated and untreated groups were compared using a standard t-test with a significance level of 0.05.

Results

PRSE analysis

The protein structure of PRSE Spirulina powder and the phycocyanin laboratory reference showed similarities in the presence of the phycobiliprotein complex. Figure 1 displays PRSE and phycocyanin laboratory reference specimens; both had fewer higher molecular weight proteins than the natural Spirulina powder specimen (Nutrex Hawaii), indicating a higher level of protein purity. PRSE contained several lower molecular weight proteins not present in the phycocyanin laboratory reference, representing differences in the extraction and purification processes.

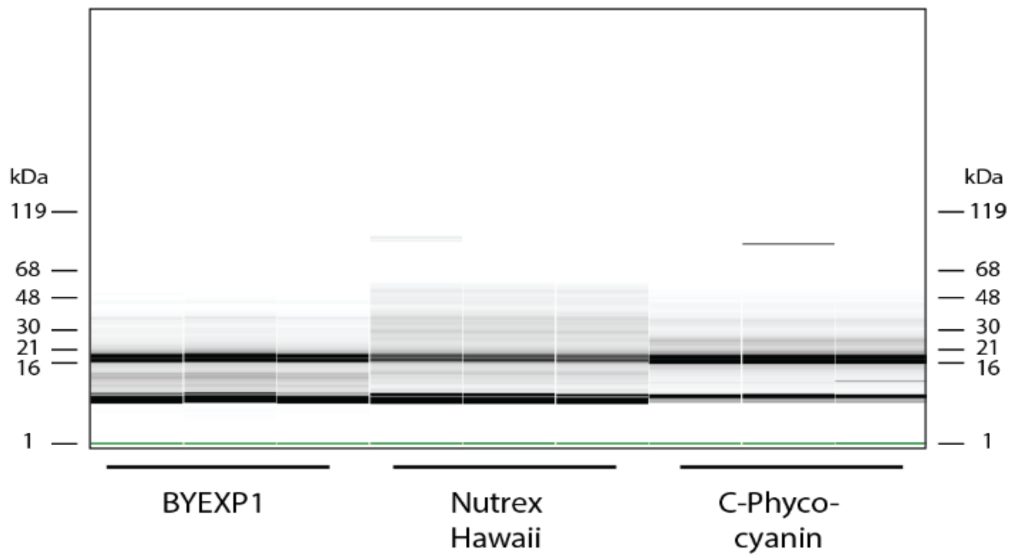

Figure 1: LabChip molecular weight profiles of PRSE (BYEXP1), Spirulina powder (Nutrex Hawaii) and phycocyanin laboratory reference (C-phycocyanin)

Growth, phenotype, yield, photometry and photosynthesis

The PRSE-treated lettuce (treatment group) reached maturity and was harvested at 22 days, which was 6 days prior to the harvest of the untreated lettuce (control group). The treatment group showed a mean increase of 2.6cm in leaf length and 2.2cm in basal stem diameter compared to the untreated lettuce. The accelerated growth of PRSE-treated lettuce was accompanied by a 12.5% (P<0.05) increase in yield.

	Treatment	**Control**	**P value**
Leaf Length (cm)	12.8	10.2	<0.05
Basal stem diameter (cm)	6.5	4.3	<0.05

Table1: Mean leaf length and stem diameter at harvest (22 days for treatment and 28 days for control group)

The PRSE-treated lettuce was 17% brighter (L*) and 75% greener (a*) than the untreated group with a 65% improvement in Maximum Quantum Yield and a 22% improvement in Performance Index photosynthetic parameters.

	Treatment	**Control**	**P value**
CIELAB (L*, a*, b*)	42,-3,22	35,-12,2	<0.05
Maximum Quantum Yield	6.5	2.3	<0.05
Performance Index	1.6	1.4	<0.05

Table 2: CIELAB, Maximum Quantum Yield and Performance Index at harvest (22 days for treated and 28 days for control group)

Quality

Twelve clamshells of treated (harvested at 22 days) and control (harvested at 28 days) lettuce were tested for shelf life. Wilting and loss of color was seen 2-3 days earlier in the untreated group. Three samples from the PRSE-treated and untreated groups were tested for the flavonoids and showed a mean increase of 30% in Quercetin and a 8% increase in Luteolin. Blinded visual inspection and organoleptic evaluation indicated that post-harvest, PRSE-treated lettuce had better texture, stronger aroma, more intense flavor, and better mouthfeel than the untreated group.

Discussion

We conducted a scoping, controlled trial to identify and quantify the effects of PRSE on hydroponically cultivated lettuce. The trial yielded initial evidence that PRSE has a biostimulant effect, improving growth, yield, and quality. The PRSE-treated lettuce was more robust, more vigorous and reached maturity 21% more rapidly than the untreated group.

Shortening the time between planting and harvest reduces energy requirements and labor costs in VF. While it is possible that current large-scale VF operations operate with an optimized growing environment, a shortened growing time (even less than 24 hours) may have important implications for profitability. A critical consideration remains energy consumption, where for all forms of agriculture (in appropriate geographies) outdoor farming may be a more economically (and possibly environmentally) sustainable option when compared to VF [Mills and Zeramby 2020]. Given that outdoor-farmed leafy greens are 3-5 times less expensive to grow than similar vertically-farmed crops, reduced growing periods may have important implications for the economic viability of VF [Tasgal 2020]. While PRSE did improve yield, the economic impact of this finding on VF may be secondary to improved growth velocity, as VF yield is substantially influenced by the skill, lighting, and environmental conditions. The availability of biostimulants such as PRSE may assist in extending VF into the production of food staples such as wheat, corn, and rice. While excellent yields can be obtained in indoor wheat vertical farms, there is a need for innovative solutions to reduce energy consumption [Asseng et al. 2020]. The improved color, vigor, organoleptic and nutritional properties, and the longer preservation of the PRSE-treated lettuce may play a vital role in ensuring better selling prices, thereby also improving the economics of VF. Because flavonoids are an important group of polyphenol antioxidants, increased levels in the PRSE-treated group are important in ensuring that indoor cultivated lettuce offers similar or better nutritional quality to outdoor grown lettuce [Kim et al. 2016].

There is still considerable uncertainty as to the mechanism of action of natural biostimulants on growth, yield and nutritional quality [Francesca et al. 2020]. Biostimulant phytohormones (including auxins and brassinosteroids) are present in the extracts of certain microalgae species, but their levels in Spirulina are regarded as low [Haroussia et al. 2016]. The immediate and linear effect of PRSE, its effect on the photosynthetic parameters and its activity at extremely low doses may support the hypothesis that there is some cellular level activity. It is possible that PRSE can indeed improve photosynthetic efficiency at a cellular level, perhaps even acting in some way on the glycolate pathway. It is also possible that phycobiliproteins play some role linked to a core photosynthetic process — fluorescent resonant energy transfer (F.R.E.T.) [Lerer 2019]. Some support for this hypothesis can be derived from the finding that the addition of functional cyanobacterial (blue-green algae) components into plant chloroplasts improves photosynthetic efficiency including through C3 RuBisCO suppression [Price et al 2013] [South et al. 2019]. PRSE proteins have emulsifying properties and their degradation products may be a nitrogen and sulfur source, thereby acting through improving plant nutrition [Decessaro et al. 2017]. PRSE is strong promoter of bacterial activity and we are exploring its potential as a bioremediation agent [Kamaleson et al. 2020]. As plant growth promoting microorganisms (PGPMs) in the rhizosphere of hydroponically-grown plants play an important role in improving growth and vigor, the microbiome stimulation properties of PRSE warrant further research [Sheridan et al. 2016] [Lee and Lee 2015].

Limitations of this study include the small sample size and the limited number of replicates. Further studies will be undertaken to quantify a number of observations, such as the substantial root volume and mass increase observed in the treated group. Additional research is also required to fully elucidate the molecular mechanism of action of PRSE especially as it pertains to its role in improving nutritional quality [Kim et al. 2016]. We have field trials underway in established vertical farms to investigate whether the application of PRSE increases the prevalence of food borne pathogens [Coleman et al. 2017]. It is also important to consider whether the growth velocity, yield, and quality benefits derived through the use of biostimulants such as PRSE justify their price, given that PRSE constitutes less than 15% of the algae biomass and that extraction and purification steps are required. Further analysis is required, especially in vertical farms that are operating at near-optimal photosynthetic and nutritional efficiency, where the incremental growth velocity and yield benefits of biostimulation may be small. It is possible that improved nutritional quality and shelf life (positively effected by PRSE) may be of greater importance to large-scale growers than for example, a small increase in yield.

Conclusions

The long-term economic, environmental, and social impact of VF will be largely determined by its economic sustainability [Goodman and Minner 2018]. This investigation showed that the application of PRSE enhanced growth speed, yield, and quality in hydroponically grown lettuce. While further research is required, PRSE may be an important and innovative production input contributing to the economic sustainability of VF. Besides showing the potential of PRSE to reduce growing time thereby saving energy, we provide initial evidence that PRSE improves product quality (appearance, nutritional density, shelf life, and organoleptic properties). The availability of effective biostimulants supports efforts to use VF to enhance food security in areas with limited farmland and adverse environmental conditions. In these areas, VF may enable the cultivation of food staples, including important row crops such as wheat and corn. This study of the application of PRSE in VF also provides some early support for broader consideration of the role of combinations of microorganism extracts including bacteria, mycelia, and mycorrhizae as biostimulants in VF.

Declaration of interests LL and CK are employees of Back of the Yards Algae Sciences LLC and LL holds a published patent relevant to this work.

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