Improving the growth conditions of herbs to increase their potential value in vertical farming systems.

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Abstract

Global crop production is endangered by multiple environmental factors including weather, insufficient water supply, soil degradation and land availability. This is burdening field agriculture and limiting production per unit area and has the potential to not meet the survival requirements of the rising population. To meet this demand, other options for food production need to be explored that have reduced environmental reliance and impacts. Plant factories or vertical farming systems overcome most of the drawbacks of field production. Crops in these systems can be produced all year round and have a longer shelf life. They are completely pesticide free and higher in nutritional value and in many cases reduce transport cost from farm to consumer. Additionally, if using hydroponics or aeroponics growth systems, the production can be completely soil free. However, the major cost in these systems is electricity usage with the majority of this consumption by the LED lamps and temperature control systems with the remaining consumption required for maintaining resource efficiency, controlling water usage and the other environmental factors. This results in the increase of overall costs for the crops produced through these systems. While maintaining approaches for an economical crop, quality is also a critical component with many factors contributing to healthy crop production, with temperature and light intensity being the most important elements. The combined effects of these two components play a major role in the nutritional quality, aroma, total harvest index and overall growth and development. In order to investigate the best temperature and light intensity for a herb to attain a desirable growth and quality, this research focuses on four herbaceous plants namely basil, mint, coriander and parsley. Optimal temperature and light conditions from the available literature was used with varied conditions for each. The differences in plant development highlight the importance of optimal conditions which can impact yield and the quality of the product. This research uses a hydroponics setup in a simulated vertical farming system to perform a comparative study with the herbs and varied temperature and light conditions combined with a calculation of lowest possible cost per kilogram of each condition of the respective herbs using an energy modelling software. This study serves as an optimisation aid for future research regarding reduction of the cost per kilogram of each herb and can be further used for staple crops to produce better quality and abundant quantity.

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Abbreviations

DW - Dry weight

EC - Electrical conductivity

EOP - End of production

FW - Fresh weight

LED - Light-emitting diode

HI - Harvest Index

PFAL - Plant factory with artificial lighting

PFD - Photon Flux Density

PPFD - Photosynthetic Photon Flux Density

PPM - Parts Per Million

TDS - Total dissolved solids

VFS - Vertical farming systems

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1. Introduction

1.1 Need for sustainable agricultural practices

One of the most challenging impacts of COVID - 19 was food security (Falkendal, T. *et al*., 2021) and there are significant challenges to feeding the global population by the year 2050 and beyond (Searchinger, T. *et al*., 2019). Additionally, there is an urgent need to introduce new technologies to protect our ecosystems while maintaining food security. Traditionally, farmers must use the resources available to them naturally, that is, soil, sun and water. Field agriculture yields a high productivity of crops in the long term but comes with a range of negative environmental impacts, which include water abstraction, soil degradation, fertilizer loss, high global greenhouse gas emissions and other practices that are harmful to the environment and climate. These approaches typically increase food security in the short term but over time, they may reduce output and threaten future production. Additionally, the largest cause of deforestation, species extinction and the irreversible destruction of habitat is land use for increasing agricultural production. With the growing global population, the demand for food is going to increase. Global crop production is expected to grow 90% through increased yields and cropping intensity, while the remaining 10% will be produced through land expansion, because of limited natural resources (FAO, 2009). It is therefore necessary to find means of producing more food with much less environmental impact at an economically viable price point. More sustainable methods are currently being developed and used to ensure a long-term viable supply and food security, including plant factories. Plant factories with artificial lighting (or PFAL) are airtight, thermally insulated production factories for plants with vertically placed layers of shelves, including electric lamps, air conditioners and circulation units, carbon dioxide supply, and a control unit for environmental conditions (Kozai, T., 2013). These plant growth systems have a productivity of over 100 times to that from field agriculture production and also provide an extended shelf life when compared to the field production (Kozai, T. *et al*., 2015). The production costs for PFAL systems are high and almost equivalent to the labor, packaging material and fertilizer costs in field agriculture. In these PFAL systems, the light equipment accounts for ~70% of the overall costs and the remainder is required to maintain the resource efficiency, as this system is constructed to lower the usage of water and carbon dioxide per unit area per kilogram and also to minimise the emission of pollutants in the surrounding environment, which is turn, consumes of electrical power (Kozai, T. *et al*., 2015). Because of these additional costs, the overall costs for the product, that is the crop, is comparatively higher than that of field production. Therefore, to enable a sustainable supply of crops, the cost per kilogram for the crop should be reduced and made more economically viable.

1.2 Vertical farming systems

Vertical farming involves growing crops vertically in a protected indoor environment, primarily using hydroponic or aeroponic cultivation systems. In addition to maximising space efficiency, reducing water usage, shortening the growing time, reducing pesticide and herbicide usage, and providing shelter from extreme weather, vertical farming offers numerous potential benefits. Vertical farming has the advantage of not requiring soil or sunlight; therefore, pests contained in soil and most abiotic stresses caused by weather conditions or and varying day lengths cannot interfere with the system (Germer, J. *et al*., 2011; Kozai, T. *et al*., 2016). In addition to being long-lasting, LED lights are cost-effective and energy-efficient, making them ideal for vertical farms. These farms have a controlled climate, and all seasons are suitable for growing and harvesting plants. The layers stacked inside the farm produce a higher yield per square meter (m^2) than traditional farming (Kalantari, F. *et al*., 2017). As the world's population is predicted to reach nine billion by 2050, 80% of whom will live in cities, this food will also feed the growing urban population (Ackerman, K. *et al*., 2014). The ability to set up vertical farms practically anywhere, even underground, allows them to facilitate hyper-local production, shortening food supply chains and supplying fresh, local food all year (Shamshiri, R. R. *et al*., 2018) and the use of fossil fuels for ploughing and seeding is eliminated with vertical farming. The growth and morphology of the crop, as well as post-harvest quality, can be significantly influenced by controlling pre harvest factors which is significantly easier in fully controlled environments (Matthies, J. P. *et al*., 1999). Vertical farms often choose leafy vegetables and herbs because they are fast-growing, short in height and of high value (Touliatos, D. D. *et al.*, 2016). Unlike vertical farming systems, the crops' quality and total yield using traditional open-field approaches completely depends on weather conditions. In the case of greenhouse production, light and temperature are the two major factors which often cannot be controlled. The temperature inside the greenhouse depends on the solar light intensity which can vary with seasons, with winter and rainy seasons giving low solar light intensity and sunny days can go from high to extreme light intensity (Kozai, T. *et al*., 2019). Despite its advantages, vertical farming also has some disadvantages, for example, due to the semiconductor junction temperature in LEDs being critical to the LED lifetime, thermal management can be energy demanding (Banerjee, C. *et al.*, 2014). In vertical farms with stacked floors, CO_2 must also be managed, which can also increase costs (Germer, J. *et al*., 2011). Additionally, vertical farms create additional costs for lighting, carbon dioxide, and water supply. There is also a limited selection of suitable plants that must meet certain criteria to be suitable, as they need to be a height of 30 cm or less for the system to fit, low light intensity and dense growing conditions, and a growing period of 10–30 days until harvest. The fresh weight of plants should be about 85% of the total weight, including roots, to be sold (Kozai, T. *et al*., 2016). The hydroponic systems do not require sunlight and soil, which is a critical factor in assessing the advantages and disadvantages of VFS.

Figure 1. A vertical farming setup using LED technology (Oh, S. *et al*., 2023)

1.3 Impacts of temperature and light intensity on herb production

Light intensity and temperature are known to be the main contributors to the total cost of production of herbs and crops in controlled environment agriculture. Unlike conventional farming, in vertical farming, the interaction of these two factors can be varied which can result in reducing the cost per kilogram of production and influence the morphological features of the crop. Several factors are strongly influenced by light intensity, including plant growth, yield, and dry matter content. Photosynthetic photon flux density (PPFD) is defined as the intensity of light that is used during photosynthesis ranging from 400-700 nm (McCree, K. J., 1972; Poorter, H. *et al.,* 2019). An increase in light intensity generally results in an increase in net photosynthesis, which increases fresh mass and yield of plants. Additionally, an increase in light intensity also increases soluble sugars which are present in the dry matter. Other plant processes, for example, transpiration, germination, respiration, and flowering are influenced by the temperature. With an increase in the temperature, the rate of photosynthesis also increases. When combined with day length, temperature also influences reproduction in leafy plants. The primary aim of crop and herb production in a commercial outdoor agricultural system is yield, which includes the dry mass of the leaves this is followed by secondary factors such as the morphology which comprises the length of inter-nodes, leaf distribution and their carbon partitioning and sometimes flavour. To obtain a completely controlled production of crops and the desired morphology, studies of their response to varying light intensity and temperature are needed. In the past, this focus on maximising the yield has driven almost all publicly available research, rather than examining the optimal conditions to achieve the lowest cost per kilogram which is desirable in a controlled environment farming as simply increasing the light intensity also increases the cost of production and sustainability can only be achieved by reducing the cost of farming. By reducing the cost per kilogram of growing in a controlled environment, sustainable agricultural production can be achieved. The overall input costs in vertical farming are currently high with approximately 65% of the power consumption by the lighting system which controls the light intensities, and this is followed by temperature control which is approximately 30% of the total cost. These two factors are the major contributors to the overall input costs in vertical farming. Hence, to reduce the cost per kilogram of the crop, we will need to tightly control these two factors.

1.4 Herbs production using hydroponics

The growing of herbaceous plants using a hydroponic system comes with a lot of advantages. Satisfying the main requirement of vertical farming systems, they have a height of 30 cm or less to maximise production. Plants growing in these systems also need to have a compact growth pattern as the distance between the layers can be small (Kozai, T. *et al.*, 2015). Additionally, herbs also provide a sufficient year-round market demand which matches well to a continuous production system. These plants are popular for their aromas, flavors and benefits and they are rich in vitamins and antioxidants, and some have antibacterial properties. Herbs which are grown hydroponically exhibit maximised yield and healthy growth (Avgoustaki, D. D. *et al*., 2020). For this to be achieved, some factors require monitoring. For example, day length should be maintained between 14 to 16 hours per day, the humidity should be around 40 to 70% and the pH for the hydroponic solution should be between the range of 5.5 and 6.5. Additionally, ventilation is an often-overlooked parameter that is important for maintaining local carbon dioxide concentrations and preventing fungal diseases. For this research project, following four herbs were chosen:

Basil

Ocimum basilicum L. is a popular aromatic herb grown for its flavour and fresh mass (Maness, N., 2003). It is one of the most widely grown herbs in hydroponic systems. Extreme high or low temperature can decrease yield and damage the herb. Basil growth usually slows down during winter, spring and autumn as the temperature is lower. While temperature impacts the rate of growth, light intensity also influences the growth. Pennisi, G. *et al*. (2020), Dou, H. *et al*. (2018) and Beaman, A. R. *et al*. (2009) have shown that the most optimal PPFD for the growth of basil based on yield is 250µmol m^{-2} s^{-1}, 224µmol m^{-2} s^{-1} and 500µmol m^{-2} s^{-1} respectively. Similarly, Larsen, D. H. *et al*. (2020) conducted a study, growing basil under increasing far-red light intensity and PPFD and showed that seed germination occurred at 300µmol m^{-2} s^{-1} in 15 days under red-white LED light. After the transplantation, 300 µmol m^{-2} s^{-1} PPFD was used for 15 days followed by five days prior to the day of harvest and an increase in PPFD of the far-red light by 0 µmol m^{-2} s^{-1}, 50 µmol m^{-2} s^{-1} and 100 µmol m^{-2} s^{-1} in addition to the 300 µmol m^{-2} s^{-1} of the red, white LED light. The resulting PFD of this treatment came up to 300, 350 and 400 µmol m^{-2} s^{-1} at 400-800nm and a 16-hour day length was used. It was also shown that the plant's dry matter was reduced under a high fraction of blue light, hence, it is not desirable for growth of basil plants as the leaves are the part which is consumed. Together they showed that the addition of far-red light for basil during development is more beneficial when added as EOP treatment pre harvest and in a lower dosage at a higher PPFD as it increases the dry matter content of both leaves and stems. In another experiment, the response of basil to different duration of far-red light before the harvest was investigated. Seeds germinated under 150 µmol m^{-2} s^{-1} red-white LED light for 10 days. After transplantation, the plants continued to grow under 150 µmol m^{-2} s^{-1} red-white light for another 21 days. Additional far-red was applied for 1 week (as EOP treatment) or 3 weeks (throughout the growth). This resulted in treatments with a total PFD of 152, 330, 330 µmol m^{-2} s^{-1} (400–800nm). The day length was 18 hours. Therefore, the overall combination of initially raising the plants at a PPFD of 150µmol m^{-2} s^{-1} and an EOP of 300µmol m^{-2} s^{-1} is suitable for the optimal growth conditions. Along with light intensity and spectra, temperature plays an influential role in the growth of basil. A study was conducted where a range of temperatures starting from 22˚C to 30˚C was used. As the temperature increased, a higher yield of basil was observed but the shelf life decreased at the same time (Bergh, R., 2019).

Mint

Mentha spicata is another significant and widely used herb on a global scale, that is in demand all year-round. Mint has an advantage as it is extremely easy to scale. And there are several types of mint: peppermint and spearmint being the most important of all. With Japanese mint *(Mentha arvensis)*, a study was conducted where both day and night temperature were used to observe any variations in growth. Though there was no significant variation observed between the temperatures, when the day temperature was set to 30˚C, maximum stem, leaf, and root dry mass was yielded

(Hussey, A. R., 1965). Not only the yield but also essential oils were observed to be influenced to give a good yield under this temperature (Duriyaprapan, S. *et al*., 1986). Presently, there is no available research on the impacts on mint by varying the light intensity in a vertical farming system.

Parsley

Parsley (*Petroselinum crispum*) is a widely used herb for flavouring and garnishing food. After basil, it is the second most suitable herb to grow in a vertical farming setup. Parsley can be harvested from vertical farming systems throughout the year. It also allows maximized yields with increased levels of acids and aromatic oils up to 20% to 40% and lower pesticide use is required when compared to soil grown parsley. According to the results from previous experiments conducted on parsley with varying light intensities, an increased yield of fresh mass was reported with increasing intensity of light. The seedlings were seen to have a 12% increase in the total fresh mass when grown in 100μmol m^{-2} s^{-1} light intensity but the seedling compactness was seen to be tangled and floppy. This was overcome by increasing the light intensity which resulted in compact seedlings (Walters, K. J. and Lopez, R. G., 2020). Using this method, a higher yield of parsley with reduced cost per kilogram can be obtained. Considering the optimal temperature, the fresh mass, height, and the number of leaves increased when the range of 10˚C to 22.4˚C was used in an increasing trend of 13-fold (45 g), 3-fold (22.6 cm), and 1.3-fold (5 leaves), respectively. When the temperature was increased further up to 27.1°C, the yield and height were observed to reduce by 29% and 11% respectively with no change in the leaf number (Walters, K. J. and Lopez, R. G., 2021).

Coriander

Formally known as *Coriandrum sativum L.*, coriander is the most popular and widely consumed herb. It is known to add aroma and flavor to a wide variety of foods, as a result there is a rapid growth in the demand for good quality coriander but growing this herb in a hydroponic system is very tricky and difficult (Silva, M. G. D. *et al.*, 2020). This is due to the seeds not germinating and those which do germinate, often push the seed out of the media, leading to root lodging before this herb can be harvested. This herb does not prefer to grow in a wet or a dry environment, which can be another challenge for it to grow in this setup. Several experiments have been conducted to control this situation and to monitor the effects of varying light intensity and temperature on the growth of this herb. Nguyen, D. T. *et al* (2019) used three PPFDs of 100, 200 and 300 μmol m^{-2} s^{-1} under 20˚C, 25˚C and 30˚C respectively. The coriander was grown hydroponically with a day length of 16 hours. The total biomass was observed to be the highest with 300μmol m^{-2} s^{-1} at 25˚C. The yield and the nutritional quality can be enhanced in coriander by controlling the light intensity and the temperature, which can be applied commercially in the future.

Overall, this research project aims to find an optimum light intensity and temperature for basil, parsley, coriander, and mint to grow in a vertical farming setup where our focus is to achieve the best yield while the total cost per kilogram is lowest.

2. Material and Methods

2.1 Seed preparation and plant growth

Plant growth was carried out using the seeds of Basil, Parsley, Coriander and Mint herbs obtained from a commercial source. Initially seed viability was examined by using a moist paper towel technique by placing the seeds in a petri dish between two layers of moist paper towels. This gave us an estimated time the seeds needed to germinate. The seeds were then sterilized by adding 70% (v/v) solution of ethanol which will eliminate the foreign bacteria and parasites. This was followed by washing with double-distilled water and placing them in a sealed container. This container was transferred to a cold storage room set at 4.2˚C for a day and the moved to a warm growth room at 22˚C for another day. This allows the germination of the seed, which were then planted on the media embryo down in a simulated hydroponic solution which reproduces the conditions necessary in vertical farming systems.

2.2 Media preparation using Hoagland's growth solution and Agar

The herb hydroponic system employed in this st8udy had not previously been used in the Taylor lab and as such many parameters needed to be optimised prior to use. Commercially obtained Hoagland's modified basal salt mixture (PhytoTechnology Labs) and Agar (PhytoTechnology Labs) was used in the media preparation. Three concentrations of Agar (65%, 75%, 85%) were initially tested for the easy growth of roots with the herb seeds. Using a laboratory measuring scale, 0.163g Hoagland's and 0.65g, 0.75g and 0.85g Agar were measured and mixed in 100mL of double distilled water and then autoclaved at 112˚C for sterilization. After covering the bottom part of the black seed holders (Figure 2. c) with paper tape, using a sterilized 5mL syringe, 3 drops were poured in these tubes and held upright until the media set. After the media is dry, using a forceps, under a sterilized environment in a laminar airflow chamber each seed was transferred in one tube by placing them on the surface of the media. This was gently pressed so that the embryonic part of the seed enters the media.

2.3 Hydroponic solutions

NULIFE technologies power growth hydroponic nutrient solution A and B were used to prepare the base solution for the hydroponic system. The EC for these two growth solutions was measured to obtain a Total Dissolved Solids (TDS) value of 2.4 for a desirable growth medium. This was measured by using H198192 EC, TDS meter. The pH was maintained to be 6.0 as per the solution requirements. A 3.5mL volume of each A and B growth solution was mixed per litre of double distilled water to make up the final hydroponic solution.

2.4 Hydroponic systems

Figure 2. (a-c) The components used in the hydroponic systems. (a) Tank filled with 1.6L of hydroponic solution. (b) Tray placed on top of the tank to hold the seed holders. (c) Seed holders to retain the seed placed on the surface of the gel. (Source: Araponics© website (https://www.araponics.com/))

Commercially obtained components were used to setup the hydroponic system (Araponics, Belgium; Figure 2. (a-c)). This setup has a developmental window for mature plants and a minimal chance of algal or bacterial contamination. Along with experimental flexibility, it also offers an intermediate throughput (Conn, S. J. *et al.*, 2013) which is desirable for this protocol. A transparent plastic lid was used on top on the setup to trap humidity and avoid evaporation of the solution.

2.5 Growth conditions

The seed holders with the seeds were placed on the tray and the tip at the bottom was immersed in the hydroponic solution (1.6L solution per tank). Treatments were performed for each of the plant seeds for the four herbs and was exposed to varying conditions of light intensity and temperature. The humidity was set to 50% for every treatment with 16 hours daytime and CO_2 at 420 ppm. These seeds were first placed in a controlled growth chamber under the same conditions until germination and then transferred to their respective chambers with the required conditions for growth. The PPFD and the temperature used were suggested from the available literature and initial research. Table 1 and 2 shows the values of light intensities and temperature used on all the triplicates respectively:

Table 1. The selected values of light intensities as PPFD (μmol m^{-2} s-1) for the herb treatments.

Herb	Treatment Number	PPFD (in μmol m^{-2} s-1)
Basil	1	400
	2	450
	3	500
Parsley	1	100
	2	150
	3	200
Coriander	1	250
	2	300
	3	350
Mint	1	250
	2	300
	3	350

Table 2. The selected values of temperature (℃) for the herb treatments.

Herb	Treatment Number	Temperature (in ˚C)
Basil	1	20
	2	25
	3	30
Parsley	1	17
	2	22
	3	27
Coriander	1	20
	2	25
	3	30
Mint	1	20
	2	25
	3	30

2.6 Monitoring the growth for yield and biomass

Plant height was monitored every two days from germination and level of the hydroponic solution in the tank was maintained throughout the treatment. Extra growth and plant litter was eliminated from time to time. After the herb has reached maturity, the total biomass including the dry and wet weight was measured. The wet weight contains the moisture content, and the dry weight informs us about cell differentiation and elongation. For the fresh weight, leaves, stems, and roots were separated and weighed individually to calculate the harvest index and further they were dehydrated and weighed for dry weight.

2.7 Determining Chlorophyll concentration

Chlorophyll content in each herb was measured for the fresh leaves using atLeaf CHL BLUE (Version 1.3) chlorophyll meter after the growth period was complete and recorded and assessed as proxy for plant health.

2.8 Calculating the lowest cost per kilogram for each of the herbs

To maintain and monitor the environmental conditions in the vertical farming setup, we will be using a simulated HVAC (heating, ventilation, and air conditioning) system. This equipment helps to stimulate the input costs for temperature control and light intensities for all the treatments of the respective herbs. Using EnergyPlus software(Version 23.2.0), the cost per kilogram was calculated for each herb. A weather file for Perth, Western Australia was downloaded from www.energyplus.net/weather (Figure 3) and imported in the Open Studio software (Version 3.6.0). This stimulates an HVAC system which was set to run for a year and the use of energy was recorded and compared the consumption to the produce in climate regions across the world. This gives the simulation results and an estimated cost per kilogram for each herb.

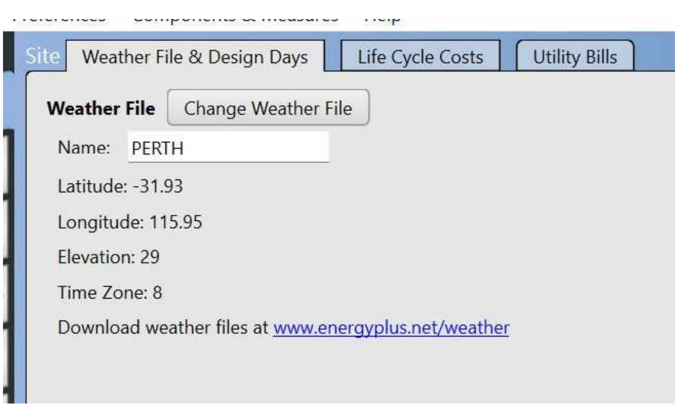

Figure 3. A weather file for Perth, WA, Australia was downloaded from www.energyplus.net/weather and imported on the Open Studio software.

A default grow room construction set was generated and following conditions were selected from the library:

Exterior Surface: Outer wall, concrete floor and roof.

Interior Surface: Insulated wall, concrete floor and insulated wall (ceiling).

Exterior Sub Surface: Exterior door.

Interior Sub Surface: Interior door.

The floor space was then built and a thermal zone and an HVAC system was added. A default schedule set was generated with 'LED on' selected for lighting and a default thermal zone was added with CLG HVAC and HTF HVAC as thermostat schedules where the temperature set points were changed as per requirements of the treatments. The energy use was then recorded for a whole year. To calculate the lowest cost per kilogram, the highest gram per PPFD was calculated for each of the herb treatments.

3. Results

3.1 Media preparation using Agar and Hoagland's solution

Three concentrations of agar was used to prepare the final media; A (65% (w/v)), B (75% (w/v)) and C (85% (w/v)). The seed holders with all three concentrations were immersed in the hydroponic solution with seeds planted in them. Tubes containing media at concentration 65% (w/v) dissolved within 48 hours in the solution and the seeds were dehydrated on the walls of the tubes as the strength of the media was not strong enough to hold the weight of the seeds and endure the heat in the growth chamber. As a result, none of the seeds in this set germinated. Set B remained intact in the setup, with the roots of Basil emerging withing seven days of planting the seeds. The roots pierced the media and exited through the outlet at the bottom of the tubes into the hydroponic solution. This concentration handled the weight of the herb easily with no further difficulties. Set C, with 85% (w/v) agar, was extremely rigid and stiff for roots to pass through it and the seed growth stalled. No further germination was seen in set C. The research project continued using an Agar concentration of 75% (w/v) Agar with 1.63 grams per liter Hoagland's solution.

3.2 Plant growth and development

The growth of the respective herbs was monitored daily during their growth. The fastest growing herb was basil which germinated in about seven to eight days. Mint and parsley germinated within 10-12 days and coriander germinated in 12-13 days. While all the herbs took the same time to start sprouting in the growth chamber with the similar conditions, there was a noticeable change in the pattern of their growth since the day they were transferred into their experimental temperatures and light intensities. Some of these differences were visual, that is root density and structural differences, leaf size and phyllotaxy or the leaf arrangement as well as aroma. Additionally, there were height differences between the herbs, chlorophyll content in the final product varied, and the fresh and dry weight were contrasting as well.

Phenotyping of herbs

Basil

Basil showed the most desirable features with the best growth occurring at 25°C with 450μmol m^{-2} s^{-1} PPFD. The roots appeared to be denser and thicker with multiple roots from each herb. As they developed, they entangled their root systems in the solution giving the herbs better strength and structural support. The leaf arrangement on each stem was denser and more regular than the other plants grown at different temperature and light intensities and the size of each leaf tended to be larger

(Figure 4). The bottom edge of each stem was thick and tough. These herbs were highly aromatic and had a deep green colour. In contrast, the basil grown at 20℃ mostly had one to three roots per herb, that were much less dense. The leaves were smaller and lesser in number with an average of four per stem and they grew in pairs on the opposite sides of the stem. There was little aroma and were in a lighter shade of green. As for the plants grown at 30℃, the stems appeared to be growing in height but the size of the leaves were of an intermediate size with a yellowish green colour with some wilting near the ends of the leaves. Roots growth was similar to that of the other plants grown at 20℃ but appeared to be a little denser with an average of five to six root lengths per plant.

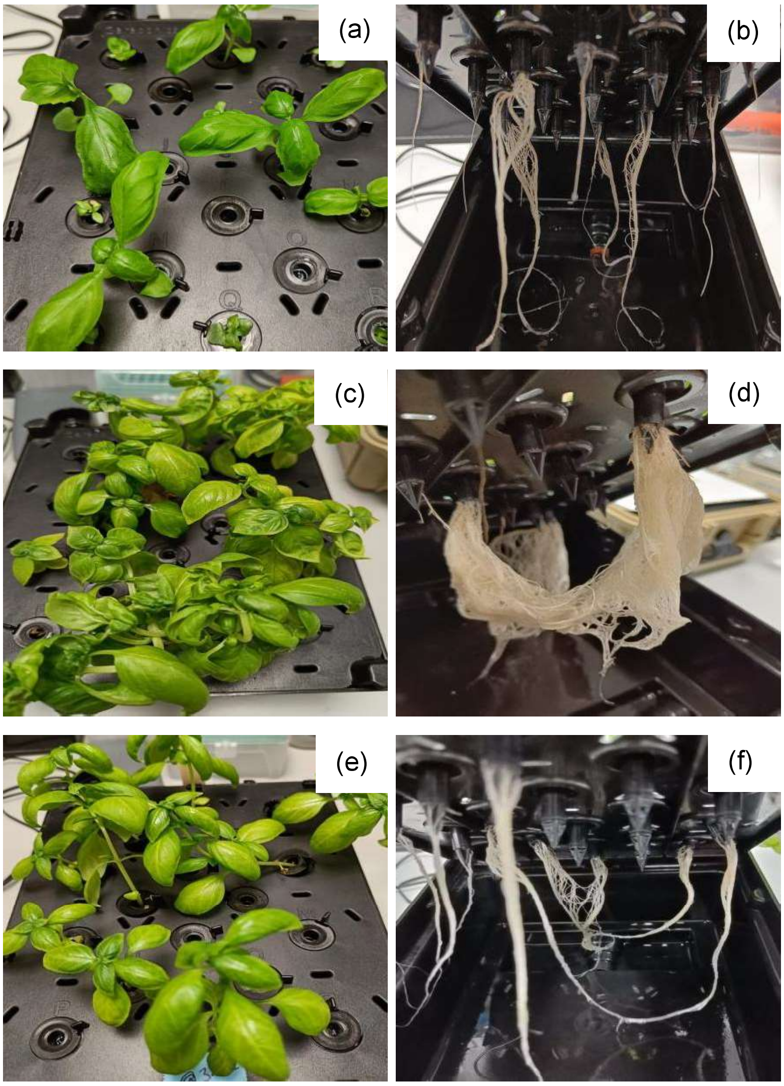

Figure 4. (a-f) The phenotypic features of Basil grown at different experimental conditions.
(a) leaves and (b) roots are the plants grown at 20℃ and 400μmol m^{-2} s^{-1} PPFD, (c) leaves and (d) roots show the plants grown at 25℃ and 450μmol m^{-2} s^{-1} PPFD, (e) leaves and (f) roots are plants grown at 30℃ and 500μmol m^{-2} s^{-1} PPFD.

Mint

At 25°C temperature and 300 μmol m^{-2} s^{-1} PPFD, mint appeared to have the best growth with the highest leaf and root density. Each stem had an average of 12 - 15 leaves with a powerful aroma and light green to green in colour. The root network appeared to be highly dense and strong. The stem elongation was the greatest in these conditions when compared to the others tested. The starting growth point of the stem from where the roots emerge was the thickest and the strongest part in the entire stem (Figure 5). As for the mint grown at 20°C, the stems appeared to be very weak and frail. The average number of leaves on the stems was seven with the size of each leaf smaller than the optimal growth regime. The roots were thin and weaker in appearance with very less density and length. Mint leaves at 30°C were smaller in size as compared to the other duplicates with frail stems and low root density. The leaves appeared to be wilting from the sides and did not have much structural integrity.

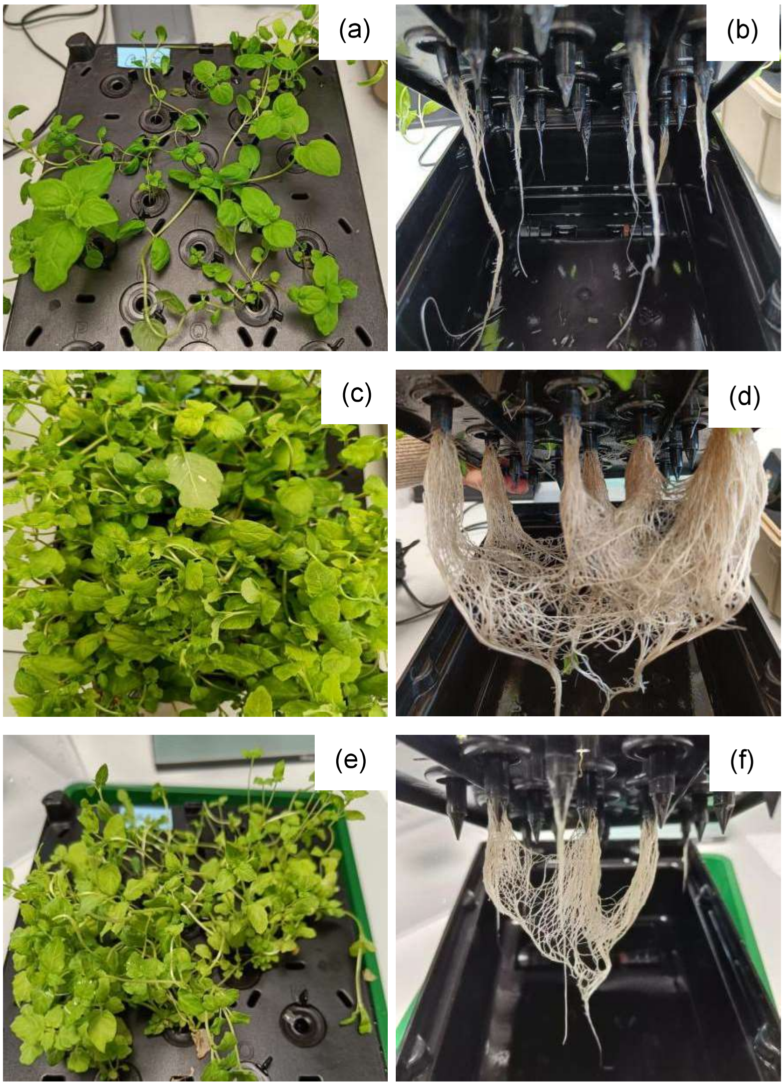

Figure 5. (a-f) The phenotypic features of Mint grown at different experimental conditions.
(a) leaves and (b) roots are the plants grown at 20°C and 250μmol m^{-2} s^{-1} PPFD, (c) leaves and (d) roots show the plants grown at 25°C and 300μmol m^{-2} s^{-1} PPFD, (e) leaves and (f) roots are plants grown at 30°C and 350μmol m^{-2} s^{-1} PPFD.

Coriander

The most aromatic herb out of the tested conditions was the coriander grown at 20℃ with 250µmol m^{-2} s^{-1} PPFD. The colour of the leaves appeared to be bright green and the height of the stem was taller compared to the other tested regimes. Each seed germinated into one stem and a network of up to five roots per seed with one coriander leaf on the tip with the most stem elongation (Figure 6).

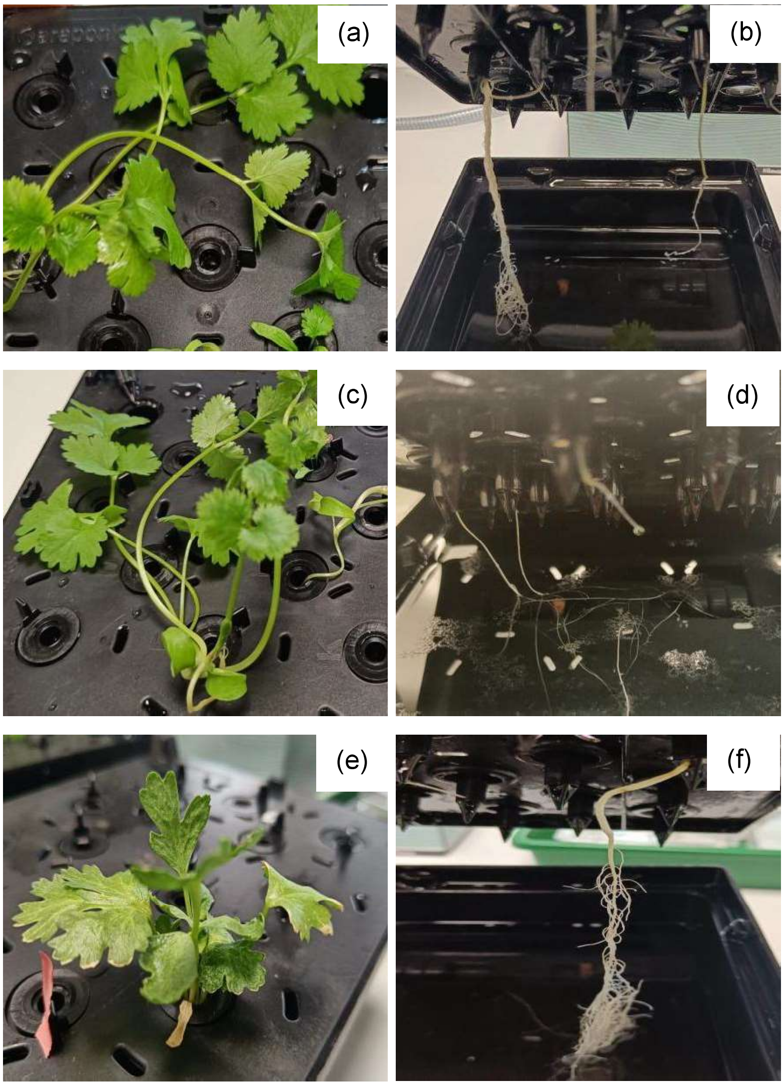

Figure 6. (a-f) The phenotypic features of Coriander grown at different experimental conditions. (a) leaves and (b) roots are the plants grown at 20℃ and 250µmol m^{-2} s^{-1} PPFD, (c) leaves and (d) roots show the plants grown at 25℃ and 300µmol m^{-2} s^{-1} PPFD, (e) leaves and (f) roots are plants grown at 30℃ and 350µmol m^{-2} s^{-1} PPFD.

Whereas, when the herb was grown at 30℃, it was a yellowish green in colour with a weak aroma. Each seed had about three thick stems, but the height was shorter as compared to the other treatments. The leaves appeared to have a hard and rough texture on their surface. Root density was weaker, and the root architecture was noticeably different. Instead of multiple roots emerging from the seed, only one root emerged and then branched on to several of them, as seminal roots. At 25℃, coriander leaves were darker shade of green and a medium aroma. However, many of the plants grown at this temperature were stunted after germination, stopping its further growth. The roots were frail and thin, and the bottom of the stems were fragile with little to no strength.

Parsley

Among the treatments, the seeds grown at 17°C and 100 µmol m^{-2} s^{-1} PPFD took the least time to grow and elongate. The leaves had a bright green colour and were the most aromatic. The root network was longer, and each seed had multiple roots emerging from it that were thicker than the other conditions (Figure 7). Parsley grown at 22°C had a good growth pattern initially, but after a seven days most of seeds stopped growing but had a green to dark green colour. The stems were thin and fragile and the root length was not more than 10 mm in most of the seedlings. Whereas, the herb at 27°C appeared to have a stunted growth after the transfer from the common chamber and were stunted at that particular stage. Many roots emerged from the seeds but the stem elongation was weak. The leaves appeared to have a yellowish green colour.

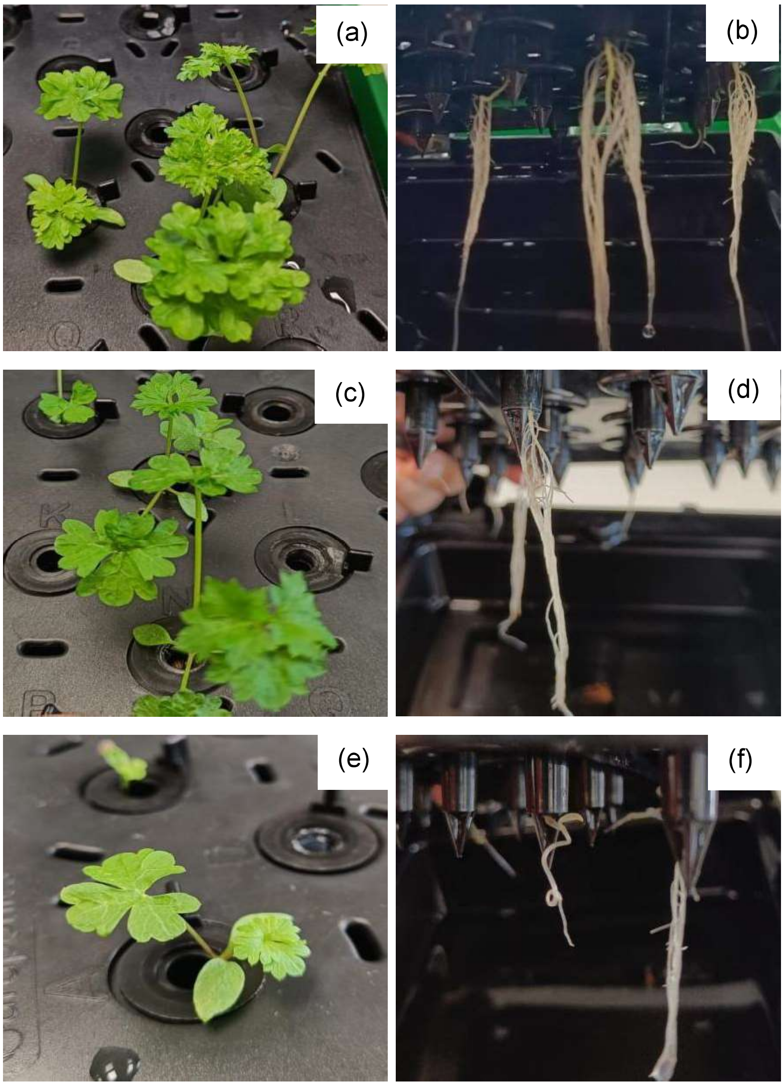

Figure 7. (a-f) The phenotypic features of Parsley grown at different experimental conditions. (a) leaves and (b) roots are the plants grown at 17°C and 100µmol m^{-2} s^{-1} PPFD, (c) leaves and (d) roots show the plants grown at 23°C and 150µmol m^{-2} s^{-1} PPFD, (e) leaves and (f) roots are plants grown at 27°C and 200µmol m^{-2} s^{-1}

Plant height

The height of the stem for each herb treatments were monitored and recorded every two days using a centimeter scale. This was done to compare the differences in growth of every triplicate at their specific conditions of temperature and light intensity.

Basil

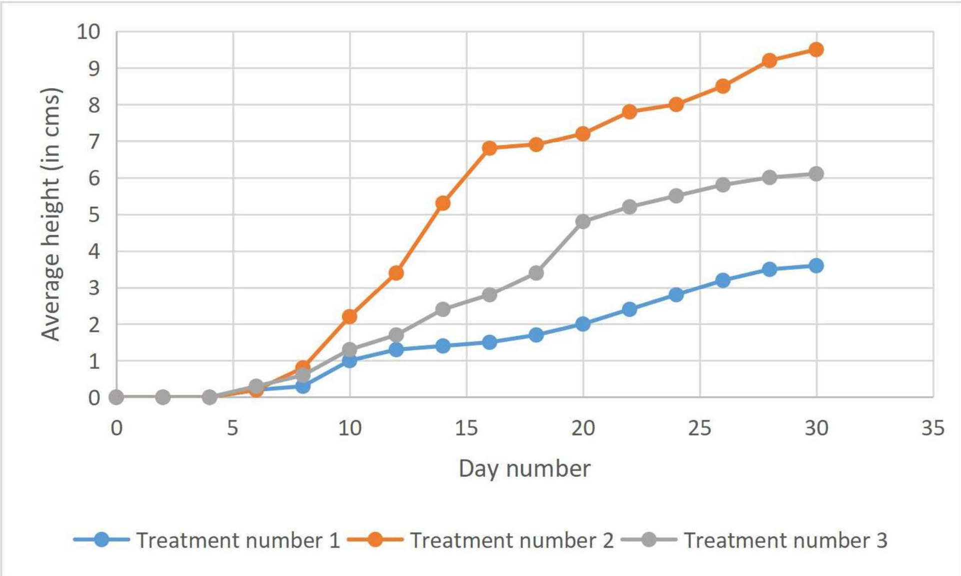

Figure 8. The average height during development for Basil treatments

For treatment 1, these plants achieved the maximum average height of 3.6cm during the first 30 days since the seeds were planted. According to the graph, the maximum growth is recorded between day 15 to 28. In this setup, just over 50% germinated seeds achieved full growth, remaining reached up to an average height of 1.2cm. For treatment 2, the maximum average height achieved by these plants by the 30th day was 9.5cm. This was the highest among all the plants grown under different experimental regimes and about 90% of the seeds produced elongated, thick stems and achieved full growth. The maximum height surge was observed between day 10 and 20. For treatment 3, about 60% of the germinated seeds achieved full growth with a maximum average height of 6.1cm during the first 30 days in this plant. It was observed that the herb gained the maximum growth between day 12 and 22. Remaining herbs which did not grow completely, appeared to have stopped elongating at an average height of 2cm.

Mint

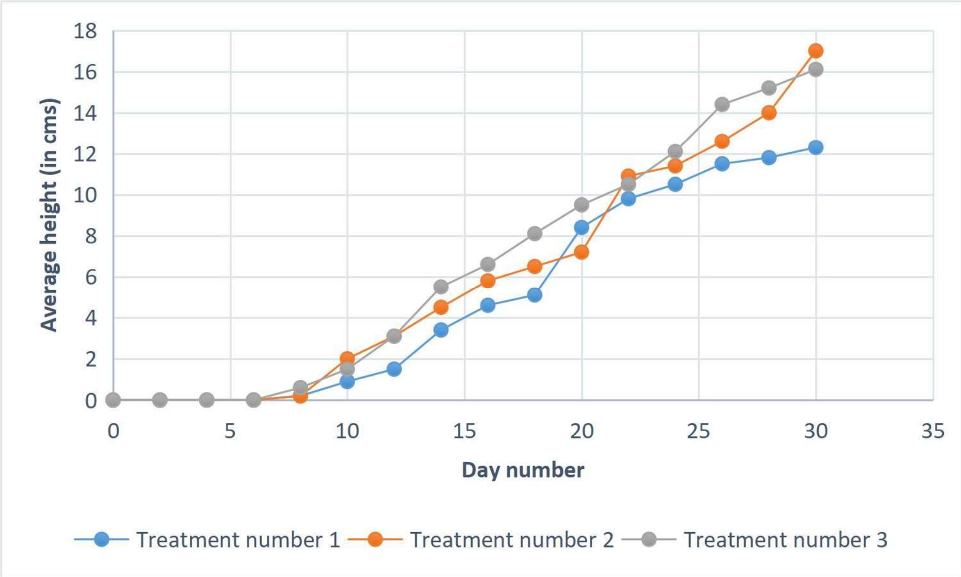

Figure 9. The average height during development for Mint treatments

For treatment 1, out of the 18 seeds that were planted and germinated, only about 40% attained complete growth in the first 30 days with a maximum average height of 12.3cm. Even though a desired stem elongation was observed, they appeared to be frail and slender, which compromised the structural integrity of the herb. The remaining 60% germinated seeds were observed to have ceased their growth after an average height of 5cm. The maximum growth for these plants was observed between day 16 and 26. For treatment 2, these plants setup produced the maximum average height of 17cm in the first 30 days with 98% germinated seeds attaining complete growth. The stem had a strong structural integrity and thick base. The maximum growth surge was observed between day 15 and 30. Out of all the other plants under different conditions, this setup gave a profitable produce and showed a desirable growth pattern. For treatment 3, in the first 30 days, these plants attained a maximum average growth of 16.1cm with 70% germinated seeds achieving a full growth. The maximum growth was observed between day 12 and 26. The remaining 30% seeds acquired stunted growth after an average height of 5.8cms.

Coriander

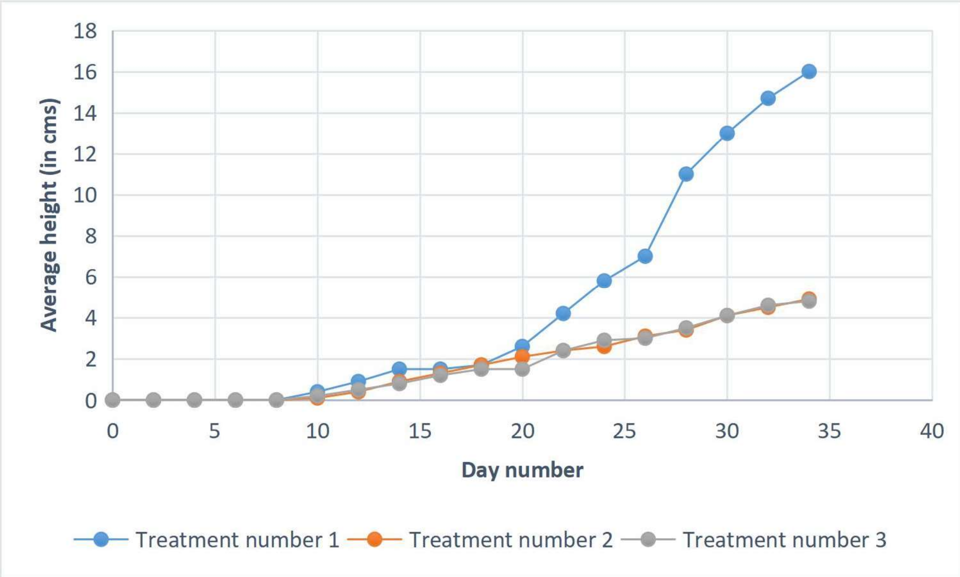

Figure 10. The average height during development for Coriander treatments

For treatment 1, the maximum average height attained in these plants was 16cm in the first 34 days with the major growth taking place between day 24 and 34. Only about 40% of the coriander seeds germinated and achieved full growth, while the remaining 60% seeds appeared to be stunted. For treatment 2, in the first 34 days of growth, a maximum average height of 4.9cm was measured in this treatment. Between the day 20 and 30, the growth was observed at maximum. Out of the 18 planted coriander seeds, only 35% germinated and attained full growth. The remaining seeds either did not germinate or stopped growing further after root formation. For treatment 3, compared to the other plants growing under different conditions, this setup achieved the lowest average maximum height of 4.8cm by the 34^{th} day of growth. Only about 30% of the planted seeds germinated and achieved maximum growth. Even though the stem elongation was not desirable, the thickness of the stem gave the herb structural integrity. Between days 18 and 20, there was no visible growth and the average height remained to be the same, and the maximum growth was observed between day 26 and 34.

Parsley

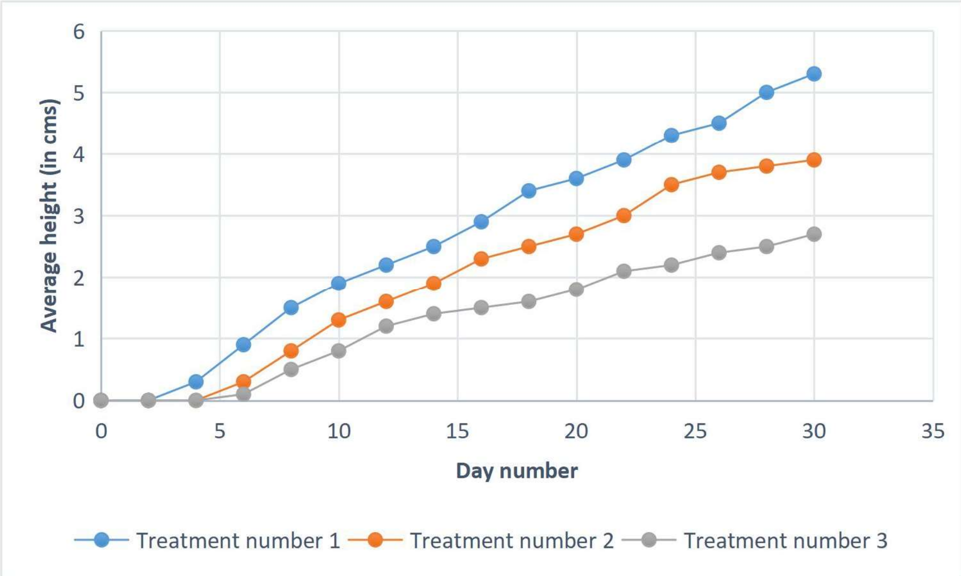

Figure 11. The average height during development for Parsley treatments

For treatment 1, plants grown under this condition attained a maximum average height of 5.3cms in 30 days, which was the highest among the other conditions, where the maximum growth was observed between day 15 and 25. In this treatment, only about 40% of the total seeds achieved full growth, where the remaining 60% germinated seeds stopped further growth after root formation. For treatment 2, these plants had the maximum average height of 3.9cms with maximum growth taking place between day 16 to 26 with about 40% of the total seeds completing their growth duration after root formation. For treatment 3, these plants acquired their maturity gradually in 30 days with maximum average height of 2.7cms, which was the lowest among all the other plants at different conditions. Only 10% of the total planted seeds grew completely after the root formation, where remaining were stunted.

Herb chlorophyll content

Using the atLeaf CHL BLUE chlorophyll meter (FT Green, https://www.atleaf.com), leaves of each herb grown in their respective conditions were measured for their chlorophyll content. Table 3 below shows the average content of chlorophyll in all the conditions:

Table 3. The chlorophyll content measured in the leaves by the atLeaf chlorophyll meter for all the herbs.

Name of the herb	Treatment number	Average Chlorophyll Content (mg/cm^2)
Basil	1 (At 20°C)	35.94
	2 (At 25°C)	37.12
	3 (At 30°C)	29.8
Mint	1 (At 20°C)	43.48
	2 (At 25°C)	39.24
	3 (At 30°C)	23.7
Coriander	1 (At 20°C)	35.53
	2 (At 25°C)	35.18
	3 (At 30°C)	35.07
Parsley	1 (At 17°C)	30.9
	2 (At 23°C)	41.1
	3 (At 27°C)	38.4

Herb fresh weight and dry weight

After reaching the full growth period, each herb was taken and leaves, roots and stems were separated. Each component was weighed for fresh weight on a laboratory weighing scale. The scale was calibrated to zero before starting the weighing process for each component. After noting the fresh weight, the herb components were dehydrated in an oven at 72°C until moisture content in the components reduced and weighed for their dry weight.

Basil

For treatment 1, at the start of the experiment, 18 seeds were planted on the gel, but in the case of this plant setup, only 50% were able to produce measurable quantities. The total DW was approximately 4% of the total FW for the roots and 5.8% for stem. The average fresh and dry weights for the leaves were 0.402g and 0.032g respectively with total DW being 8.1% of the FW. For treatment 2, a 90% germination was observed with the highest weights among all the plants grown at different conditions. The dry weights in roots and stem were 4.9% and 22.7% of the fresh weights respectively. As for the leaves, the average FW (4.32g) and DW (0.41g) were measured to be highest with the percentage of dry weight being 9.3% of the fresh weight. For treatment 3, with the 60% seedlings developing, the average fresh and dry weights were measured to be 0.70g and 0.05g respectively for the leaves. The percentages for DW compared to the FW were 3.8%, 7.8% and 7.5% for the roots, stem and leaves respectively. Figure 12 below shows the components after being biologically fractionated.

Figure 12. All the components of Basil plant biologically fractionated and placed in plates in the following order: roots, leaves, stems.

Mint

For treatment 1, about 40% of the total seeds grew into plants in these conditions with percentages of total dry weight with fresh weight to be measured as 3.2% (roots), 5.9% (stem) and 10.6% (leaves). The average fresh and dry weights for leaves were 0.17g and 0.02g respectively. For treatment 2, almost all the seeds (99%) planted in this setup germinated and attained a full growth. The average FW and DW for the leaves was measured to be 3.76g and 0.36g respectively with total dry weight being 9.5% of the total fresh weight. As for the roots and stem, this percentage was 3.9% and 7.5% of the total FW respectively. These plants gave the highest amounts of total fresh and dry weight as compared to the other basil plants grown at other conditions (Figure 13). For treatment 3, an overall germination of 70% seeds was achieved in this herb treatment. The total dry weight for roots was

4.5% and for the stem were 5.8% of the total fresh weight. As for the leaves, this percentage was 8.7% with an average dry weight of 0.05g and average fresh weight of 0.47g.

Figure 13. All the components of Mint plant were biologically fractionated and placed in plates in the following order: stems, roots, leaves.

Coriander

For treatment 1, only about 40% germination was obtained which was the highest among these the coriander seeds grown under different treatments. An average dry and fresh weight of the leaves was measured at 0.052g and 0.428g respectively with the total DW being 12.2% of the total FW. In roots and the stem, the percentage of total dry weight in fresh weight was measured as 2.8% and 24% respectively, which was the highest in coriander. For treatment 2, a 35% measurable produce was observed in these plants where the average fresh weight and dry weight was 0.4g and 0.08g for the leaves. The percentage of total dry weight in the total fresh weight was measured as 2.3% for the roots, 23.4% for the stem and 21.3% for the leaves. For treatment 3, out of all the planted seeds, only about a 30% germination was obtained, which was the lowest total produce among the three coriander plants grown in different conditions (Figure 14). The total dry weights of roots, stem and leaves were 5.7%, 24.8% and 21.9% of the total fresh weights respectively. Here, the average FW was 0.5g and DW was 0.1g for the leaves.

Figure 14. All the components of Coriander plant were biologically fractionated and placed in plates in the following order: roots, stems, leaves.

Parsley

For treatment 1, more than 65% of the seeds germinated and attained root growth but after the second week, most of the seedling developments was stunted, which was the highest in the three treatments of parsley. The seedlings which were able to grow and mature had a total DW of approximately 31% of the total FW of the roots, 39% to that of the stem and 17.5% for the leaves. The average fresh and dry weight for the leaves were 0.210g and 0.04g respectively which the highest among all the treatments (Figure 15). For treatment 2, 50% seeds germinated but only 40% of them developed after the root formation. The total dry weights in the roots, stem and the leaves were 3.6%, 24.6% and 12% of the total fresh weights respectively, where the average FW was 0.060g and DW was 0.008g in the leaves. For treatment 3, the average FW and DW for the leaves were 0.055g and 0.007g respectively with a total seedling development of only a 10%. Remaining seedlings were stunted post root development. The dry weights were 3% (roots), 12.5% (stem) and 13.2% (leaves) of the total fresh weights. This treatment had the least measurable quantities as most of the seeds were stunted either before germination or after root formation.

Figure 15. All the components of Parsley plant were biologically fractionated and placed in plates in the following order: stems, roots, leaves.

Yield and Harvest Index

Calculating the harvest index is a necessary step in the measurement of the crop yield. The formula for the calculation of HI in herbaceous plants is:

Harvest Index (HI %) = (Harvested product / Total weight of the herb) x 100

In this formula, the harvested product implies to the weight of the leaves and the total weight of the plant includes weight of stem, roots and the leaves. In case of herbaceous plants, some vendors also provide the stem as a selling product for consumption, so the harvested product is the sum of total weight of leaves and the stem in the following calculation. The total fresh weight values of the respective components have been used here as harvested product and total weight (Table 4).

Table 4. The calculation for harvest index (%) where the sum of total fresh weight of leaves and stem is used as the weight of the harvested product in grams.

Herb	Treatment number	Harvested Product (g)	Total weight of the herb (g)	Harvest Index (%)
Basil	1	3.49	4.24	82.3
	2	25.06	40.96	61.2
	3	8.63	10.32	83.7
Mint	1	4.62	5.46	84.7
	2	47.84	55.09	86.8
	3	7.22	8.18	88.3
Coriander	1	2.81	3.30	85.1
	2	0.84	1.22	68.9
	3	0.83	1.29	64.3
Parsley	1	0.97	1.73	56.1
	2	0.43	0.74	58.1
	3	0.08	0.18	44.4

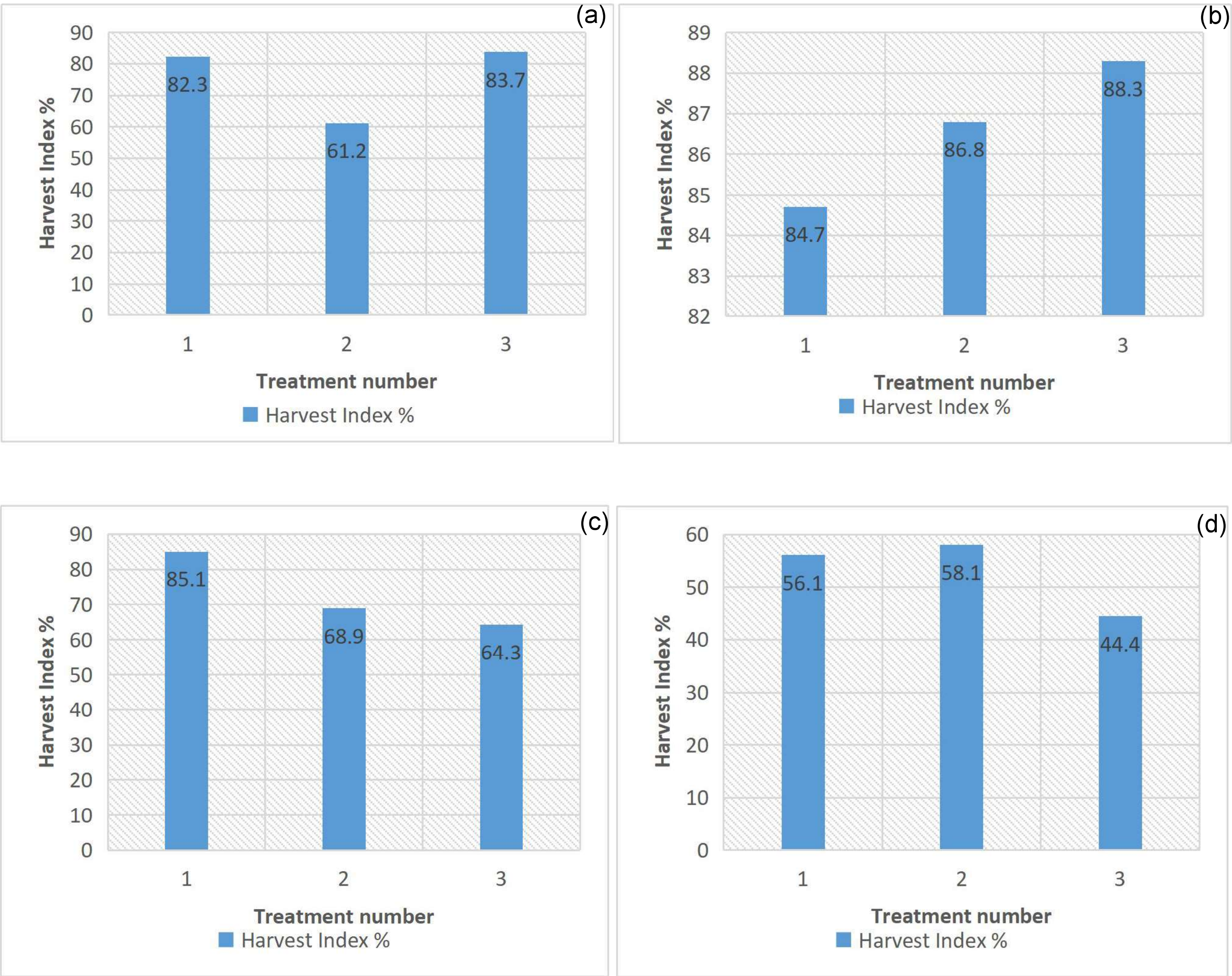

Figure 16. The harvest index percentages of (a) Basil, (b) Mint, (c) Coriander and (d) Parsley treatments

3.3 Calculating the cost per kilogram for herb production

Data from Perth and the growth condition were entered into the EnergyPlus (Version 23.1.0) software and the simulation was set to run and values were gathered for over 8760.00 hours. (One year, Table 5.)

Table 5. The site and source energy utilized by the growth room building generated by the Energyplus.

	Total Energy [GJ]	**Energy Per Total Building Area [MJ/m²]**	**Energy Per Conditioned Building Area [MJ/m²]**
Total Site Energy	25.00	1041.85	1041.85
Net Site Energy	25.00	1041.85	1041.85
Total Source Energy	79.19	3299.55	3299.55
Net Source Energy	79.19	3299.55	3299.55

Table 6. The total electrical end usage in GJ where it is the principal heating source, based on the energy usage.

	Electricity [GJ]
Heating	9.75
Cooling	1.55
Interior Lighting	10.09
Exterior Lighting	0.00
Interior Equipment	0.00
Exterior Equipment	0.00
Fans	3.60
Pumps	0.00
Heat Rejection	0.00
Humidification	0.00
Heat Recovery	0.00
Water Systems	0.00
Refrigeration	0.00
Generators	0.00
Total End Usage	25.00

The total utility use per conditional floor area and total floor area was 420.48MJ/m^2 for lighting equipment and 621.37MJ/m^2 for the HVAC system. The total electrical intensity utility was 1041.85MJ/m^2. For electric loads, total electrical end usage was 25.004 GJ in maintaining the growth room.

It can be assumed that light intensity in PPFD correlates linearly with energy use and overall production costs. For the calculation of the lowest cost per kilogram, the highest yield in grams per PPFD from the herbs is considered. The yield in grams values are used from Table 4, where the highest gram per PPFD is the lowest cost per kilogram. For basil plants, the yield (in grams) per PPFD for treatments 1, 2, and 3 were 0.011, 0.091, and 0.021 g/PPFD, respectively, and the lowest cost per kilogram was 0.091 g/PPFD from treatment 2. For mint plants, the yield per PPFD was 0.022 (treatment 1) g/PPFD, 0.184 (treatment 2) g/PPFD, and 0.023 (treatment 3) g/PPFD, and for coriander plants, it was calculated to be 0.013, 0.004, and 0.003 g/PPFD for treatments 1, 2, and 3, respectively. The highest gram per PPFD for mint plants was treatment 2, and for coriander it was treatment 1. The yield per PPFD for the parsley plant was 0.017 g/PPFD for treatment 1, 0.005 g/PPFD for treatment 2, and 0.0009 g/PPFD for treatment 3, with the lowest cost per kilogram from treatment 1.

4. Discussion

By applying PFAL for a controlled plant growth enables an absolute control of the environmental factors, including light intensity, temperature, CO_2 , humidity and nutrient solution contents (Kozai, T. *et al.*, 2015). In an uncontrolled or field agriculture, these natural resources are bound to fluctuate due to various reasons, which requires the plant to acclimatise and adjust most of their morphological traits, including total biomass, stem length, leaf area and colour. These changes also alter their overall nutrient composition. Therefore, controlled environmental conditions in plant factories are essential to produce desirable characteristics in a plant with the aim to meet the demands of the customer. The main objective of this study was to improve the growing conditions of herbs by adapting controlled environmental conditions in a vertical farming setup and using varying temperature and light intensity to monitor their impacts on plant growth, and to calculate the overall cost per kilogram of the production. The lighting system in a plant factory has the highest energy demand, followed by temperature control system. Both light intensity and temperature play a significant role in the overall plant growth and development.

4.1 Basil

Basil is the one of the most popular herbs grown in a vertical farming system using hydroponics. It was the first herb to germinate withing a week in this study. Extremely high of low temperatures impact the quality of this herb. Considering the phenotypic features, the most desirable growth was observed in the plant grown at 25°C and 450 µmol m^{-2} s^{-1} PPFD. The root system was dense and strong with thick and tall stem. The PPFD used on a plant enables photosynthesis, which accelerates sucrose production (Van Gederen, K. *et al*., 2018), facilitating root growth and development. A lower PPFD resulted in poor root growth and shorter stem. Burko, Y. *et al.,* (2022) conducted a study on the effect of temperature and light together on plant growth, stating that colder temperatures and higher light intensities together slow down the stem growth, as seen in this study. Increased light intensity also correlates with increased yield and fresh mass. The highest percentage of harvest index was observed in the plant grown at 500µmol m^{-2} s^{-1} PPFD. Zhao, Y. *et al.,* (2020) demonstrated that the rate of photosynthesis and chlorophyll biosynthesis in the leaves can be inhibited by lower temperatures. Basil at 25°C contained the highest chlorophyll content as compared to the plant at a lower and a higher temperature.

4.2 Mint

Effect of varying light intensities on a mint plant *(Mentha arvensis)* were investigated by Deraman, D. S., *et al.,* (2018). They observed that the plant height grows faster under low light intensity and the shade period when increased, results in a significant increase in stem length. In this research, we provided a shade period of eight hours, and the mint grown at lower temperature of 20°C and 25°C showed the maximum average height. They also stated that number of leaves on mint decrease with an increase in light intensity. The root network was strongest at 25°C and 300 µmol m^{-2} s^{-1} with the maximum number of leaves on each stem. The maximum DW and FW was also observed under these conditions. Although the total yield was the highest for this setup, the maximum harvest index percentage was observed at 350µmol m^{-2} s^{-1}. In this experiment, the maximum chlorophyll content was observed at a lower temperature which is contrary to the fact that lower temperatures suppress chlorophyll biosynthesis. This may have been caused by difficulties in the application of the AtLeaf meter to the Mint plants.

4.3 Coriander

Several environmental and genetic have a significant impact on seed germination, making growing coriander a complex process (Shafii, B. *et al.,* 2001). Coriander seeds have a higher germination rate in a lower temperature range of 20 - 25°C (Allahmoradi, P. *et al.,* 2013). More than 60% of the coriander seeds planted in this study did not germinate and acquired stunted growth. The maximum yield and harvest index percentage was obtained for the lowest temperature and PPFD, that is, 20°C with 250 µmol m^{-2} s^{-1} PPFD. The chlorophyll content between the three plants did not show a significant difference, the reason can be considered an incomplete data set, as the total yield in all the regimes were non-identical due to most of them acquiring stunted growth.

4.4 Parsley

Litvin, A. G. *et al.,* (2020) conducted a study on Parsley where they stated that these plants when grown under LED light tend to have high concentrations of essential oils, myrcene accumulation and flavonoids. Parsley growth slows down with increase in temperature and attains a lower dry weight than that which are grown in lower temperatures (Eidsten, I. M. *et al.*, 1986). The strongest root network and highest total biomass was observed in treatment 1, with 17°C and 100 µmol m^{-2} s^{-1} PPFD in this study but more than 70% of the total seeds did not mature after root formation. The average chlorophyll was recorded to be lower that the other conditions, this may have been caused by the difficulties in applying the AtLeaf chlorophyll meter. The maximum HI% was observed in treatment 2 (58%) and 1 (56%). At the highest temperature, maximum seed development was stunted with the least total biomass and harvest index percentage.

4.5 Production costs and electrical usage by the facility

The major challenge in vertical farming systems is the electrical consumption costs which influences the total cost of the end product (Beacham, A. M. *et al*., 2019). A study was conducted by Arabzadeh, V. *et al.,* (2023) to maximise the cost savings for a vertical farming system where they stated that the consumption costs for electricity can be reduced from 5% to approximately 30%. In terms of resource efficiency, these systems use minimal land, water and fertiliser (Despommier, D., 2013). In this study, we constructed a growth room and calculated the annual energy utilisation using EnergyPlus (Version 23.1.0) software and OpenStudio (Version 3.6.0). By minimising the production costs for plants, we can reduce the overall cost per kilogram of the herb, making it economical. However, the plants in this study with the lowest cost per kilogram cannot be relied upon as the data set was incomplete and most of the herb treatments growth was stunted. Additionally, different light intensities also impact the growth room temperature and thus the HVAC requirements. Using the data from other climatic regions, this research paper can be further used to study the interactions between the optimal conditions of light intensities and temperature for different climatic locations.

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