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The Optimisation of cultivation conditions for Basil (Ocimum sp. L) production in multi-tier hydroponics and the role of light quality in the enhancement of growth and quality

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University of Plymouth

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The Optimisation of cultivation conditions for Basil (Ocimum sp. L) production in multi-tier hydroponics and the role of light quality in the enhancement of growth and quality

By

Mohammed Imad Mohammed Aladarkazali

A thesis submitted to the University of Plymouth in partial fulfilment for the degree of

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School of Biological and Marine Sciences

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Dedication

I dedicate this work to my son, Hassan, in hope to make him proud, for all of his unconditional love, and for all the times I could not spend with him in pursuit to accomplish this research.
Author’s Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Doctoral College Quality Sub-Committee.

Work submitted for this research degree at the University of Plymouth has not formed part of any other degree either at the University of Plymouth or at another establishment.

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The Optimisation of cultivation conditions for Basil (Ocimum sp. L) production in multi-tier hydroponics and the role of light quality in the enhancement of growth and quality.

Mohammed Imad Mohammed Aldarkazali
Abstract

Basil (*Ocimum* sp. L.) is considered an important herb in Herbalism, Agronomy, Ethnobotany, Gastronomy, and Bromatology. Increases in population have led to an increase in the demands for food including Basil and thus there is an increased need to optimise its growing conditions in order to produce acceptable plants for the consumers. This optimization includes growing Basil in indoor farming situations. Basil contains essential oils which impart many of its herbal and culinary properties and whilst the chemical composition of the essential oil of Basil varied but commonly contains, Estragole, Linalool, Eugenol, E Cinnamate and Eucalyptol.

Light Emitting Diodes (LEDs) have a strong potential to enhance the growth and quality of Basil in indoor farming and might propose a general recipe for light conditions for the production of other herbs if optimised. LEDs with specific light quality; Blue 435 nm/Red 663 nm, with a ratio of 1.6 Blue light: 1 Red light, and supplying 300 µmol m\(^{-2}\) s\(^{-1}\) light intensity, promoted better yield and growth when used in a multi-layered hydroponic cultivation system compared to glasshouse grown plants. Sweet and Bush grown under LED arrays promoted better growth and quality compared plants grown under HPS lamps. Furthermore, the chemical composition of essential oil of Basil was altered (mainly Eugenol and Linalool) by light quality regulation. A combination of Blue and Red light promoted a higher yield and better quality of Basil essential oil. It was concluded that the growth and quality of Basil plants can be best controlled in a Plant Factory unit but the LED arrays for better growth still need to be optimised.

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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>$A_{\text{max}}$</td>
<td>Light saturated, instantaneous, Maximum Photosynthetic rate</td>
</tr>
<tr>
<td>CE</td>
<td>Controlled Environment</td>
</tr>
<tr>
<td>Cm</td>
<td>Centimetre</td>
</tr>
<tr>
<td>Cry</td>
<td>Cryptochrome</td>
</tr>
<tr>
<td>C</td>
<td>Compost</td>
</tr>
<tr>
<td>CP</td>
<td>Clay Pebble hydroponic media</td>
</tr>
<tr>
<td>CC</td>
<td>Coco Coir hydroponic media</td>
</tr>
<tr>
<td>DW</td>
<td>Dry Weight</td>
</tr>
<tr>
<td>EC</td>
<td>Electrical Conductivity</td>
</tr>
<tr>
<td>E&amp;B</td>
<td>Ebb and Flow hydroponic system</td>
</tr>
<tr>
<td>FW</td>
<td>Fresh Weight</td>
</tr>
<tr>
<td>FR</td>
<td>Far Red light</td>
</tr>
<tr>
<td>FL</td>
<td>Fluorescence light</td>
</tr>
<tr>
<td>$F_{v}/F_{m}$</td>
<td>Chlorophyll fluorescence rate</td>
</tr>
<tr>
<td>Gs</td>
<td>Stomatal Conductance</td>
</tr>
<tr>
<td>G</td>
<td>Green light</td>
</tr>
<tr>
<td>GC</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>HPS</td>
<td>High Pressure Sodium</td>
</tr>
</tbody>
</table>
H  Height
h  Hour (Time)
LED  Light Emitting Diode
LA  Leaf Area
LSD  Least Significant Difference
mm²  Millimetre square
mL  Millilitre
MIT  Metal Halide
Nm  Nano Meter
NFT  Nutrient Film Technique
PAR  Photosynthetic Active Radiation
PPFD  Photosynthetic Photon Flux Density
PhyA  Phytochrome A
PP  Photoperiod
PhyB  Phytochrome B
R  Red light
RH  Relative Humidity
RW  Rockwool hydroponic media
R  Raft/floating hydroponic system
UV  Ultra Violet
Chapter 1

Literature Review
**Light and light quality**

Photosynthesis is a chemical process in which light plays a major role in utilizing carbon dioxide and water, in the chloroplast, to produce glucose the source of energy for plant growth and development. In order to understand the role of different light conditions, most researchers rely on data supplied by the McCree curve (Figure 1). Dr Keith McCree experimented with the effects of light on more than 20 plant species grown in different conditions (McCree, 1972). The results of his experimentation led him to validate the spectral range of 400 to 700 nm as an adequate interpretation for Photosynthetic Active Radiation (PAR). However, McCree measured only the quantum yield of photosynthesis, quantum absorbance and action spectrum regardless of the effects of different wavelengths.

In general, the response of plants to light can be demonstrated with curves that are explained as rectangular hyperbolas with the linear portion taking place at PAR with values near 20-60 μmol m$^{-2}$ s$^{-1}$. Quantum efficiency can be explained by the amount of carbon dioxide integrated or the amount of O$_2$ released per quantum absorbed in absence of photorespiration. McCree did not calculate quantum efficiency as the initial slope of light-response curves. Based on the assumptions that photosynthetic light-response curves are described as rectangular hyperbolas and that quantum efficiency was wavelength-independent, he predicted that quantum efficiency could be estimated by a single-point measurement of the photosynthetic rate at low photosynthetic photon flux densities.

McCree demonstrated his findings in graphical format however, the values of the graphs are all relative at peak value of 1.0. The graphs combined both plants grown in growth chambers (controlled conditions) and plants grown in the field (uncontrolled conditions). The light responses in both conditions cannot be cross-compared directly and combining them in a single graph would not be accurate.

Photosynthesis is a quantum process directed by photon assimilation, however, the energy levels of photons can vary depending of different wavelengths. Moreover, a representation of the relative photosynthesis response based on photons must be able to differentiate that based on energy of those photons as shown in (Figure 1). However, manufacturers of lights (especially the newer LED lights) will often make graphical comparisons of the spectral distribution of their lights to that of the McCree Curve,
but it is important that both curves are using the same weighting technique to make a meaningful comparison. The data for the energy weighted curve came directly from the McCree’s results for controlled environment chamber grown plants. The quantum weighted curve was calculated from the energy weighted data points by applying the Plank relationship formula $E=hc/\lambda$ and as such light spectrum comparisons to the McCree curve do not imply an ideal light profile for plants.

Figure 1. McCree curve of PAR (Eyehortilux, 2019; McCree, 1981). Figure 1 has been removed due to Copyright.

Although total PAR is now commonly assumed to be in a fixed range (400 - 700 nm) plants do not respond uniformly (photosynthetically) to all wavelengths of PAR and in particular there is a proportionally greater response to the red region of the spectrum (600 - 700 nm) and violet-blue region (400 - 480 nm) corresponding to the absorption spectrum of Chlorophylls A and B. Interestingly, devices developed to measure PAR (quantum sensors) do not distinguish such differences and may therefore overestimate the true amount of PAR especially if the light source emits a proportionately high level of light in the green region (500 – 600 nm). More recent developments of hand-held spectro-radiometers may be able to help scientists distinguish differences between different artificial lights more clearly in the future.
Light Emitting Diode Lights (LEDs)

LEDs as luminaires (light bulb replacements) have undergone rapid development in the last 10 or so years and have been shown to have potential for use in agricultural and horticultural production (Bula et al., 1991; Schubert, Cho & Kim, 2000). LEDs have a more linear photon output with electrical input current than previous lights (Green et al., 2001). An early testing of LEDs in agriculture was demonstrated in 1991 which established the energy-saving aspect of LEDs when tested on lettuce (Bula et al., 1991). LEDs also have also been shown to have an important role in plant growth because LEDs can be constructed in arrays big enough to provide enough PAR but still small in size (few centimetres in diameter) and still emit less heat than “traditional” high intensity discharge lighting lamps (Bosma et al., 2017; Singh et al., 2015; Tonzani, 2009; Van Ieperen & Trouwborst, 2007). LEDs are also known for their durability and long operating lifetime (Dickinson, 2007). LED arrays can be designed to have wavelength specificity (Brown, Schuerger & Sager, 1995) which is a key factor due to the fact that each plant species differentially responds to different light wavelengths due to specific differences in their photoreceptors (Sims & Gamon, 2002). Numerous research has investigated the role of LEDs to enhance plant shape, edible quality (Blue 454 nm and Red 660 nm) (Lin et al., 2013), biomass, number of leaves (Blue 400-500 nm and Red 600-700 nm) (Goins et al., 1997), growth rate and stem width (Blue 400-500 nm and Red 600-700 nm) (Kim et al., 2004a). Simultaneously, research has demonstrated the effects of LEDs on chemical compounds such as vitamin C content, soluble sugar content (Blue 400-500 nm and Red 600-700 nm) (Ohashi-Kaneko et al., 2007), chlorophyll level (Kim et al., 2013), antioxidant activity (Blue and Red LED were 465–470 nm and 625–630 nm) (Wu et al., 2007) and different protein levels of many plant species.

Light intensity

Light intensity refers to the energy or measure of energy delivered by a particular light source. It is the proportion of the wavelength-weighted power transmitted by a radiance source per solid angle unit (Monteith & Unsworth, 2013). Light intensity is an important factor for plant growth as photosynthetic rate seems to respond to the light intensity in a directly proportional manner if other growing conditions
are fixed (Shirley, 1929) but research has shown that plants respond differently to different light intensities. Young Tomato plants showed better growth under a light intensity of 300 µmol m$^{-2}$ s$^{-1}$ than growth under a light intensity of 450 and 550 µmol m$^{-2}$ s$^{-1}$ when there was less difference of growth rate (Fan et al., 2013). On the other hand, growing tuber crops such as potato showed that the higher light intensity used the better quality of the crop (Porter, 1937) as well as for the growth of Spray Carnation Plants (Enoch & Hurd, 1977). Light intensity can play a role post-harvest for Sweet Basil (*Ocimum basilicum* L.) and it has been shown that when treated with light intensity between 30 and 37 µmolm$^{-2}$ s$^{-1}$ in storage, a delay of chlorophyll degradation is noted alongside less leaf wilting (Costa et al., 2013). Greek Basil can reach full growth under a light intensity of 21.9 µmol m$^{-2}$ s$^{-1}$ according to (Skrubis & Markakis, 1976). Where (Shiga et al., 2009) used 100 µmol m$^{-2}$ s$^{-1}$ light intensity for growing Sweet Basil for Rosmarinic acid content and antioxidant activity. (Amaki et al., 2011b) used 50 µmol m$^{-2}$ s$^{-1}$ light intensity for his analysis on Sweet Basil. This lack of consensus of what is the optimum light intensity for Basil leads to the conclusion that more studies needed in this area.

**Light for plants and seeds**

Plants are affected by light not only through photosynthetic activation but also in photomorphogenesis (light induced morphological development, photoperiodism (plant reaction to the length of dark/light), phototropism (plant growing in response to a light stimulation 400-500 nm), response to light intensity, chloroplast movements, and stomatal conductance. PAR is measured by the number of photons in the 400-700 nm range encountered by plant leaves for a specific period of time and this called the Photosynthetic Photon Flux Density (PPFD) µmol m$^{-2}$ s$^{-1}$ (Hall et al., 2013; McCree, 1981). Most PAR is absorbed by chlorophyll a & b in addition to carotenoids that play a role in light absorption and energy transfer to the photosystems (Franck & Loomis, 1949; Frank & Cogdell, 1996). Anthocyanin is another pigment that provides flowers with their colours in most plants. Anthocyanin utilized by plants as a protective mechanism agent harmful UV light and although it reacts with UV light, it found to react to other parts of PAR as well (longer wavelengths). However, colour characteristics of plants can not
exclusively attribute to Anthocyanin, for example, in Caryophyllales the colour features are a result of Betalains pigment (Lee, 2010).

Based on the literature it was established that there is a reaction between light and seeds. Blue light has been found to have a strong effect on seed germination (Das et al., 2020; Li et al., 2020) but red light has been shown to have the same effect in other species. Lettuce (Lactuca sativa L.) seeds were found to have the greatest promotional effect under red light 660 nm in comparison with 525 and 700 nm (Borthwick et al., 1952). Furthermore, it established that red light and Cytokinin (cell division Phytohormone) had similar effects on lettuce seeds (Miller, 1956). Seed germination of lettuce was inhibited under the exposure of far-red light with a wavelength of 700-820 nm and as well as under yellow light in the range of 580-600 nm. It was later confirmed that the seed embryo contains phytochromes (PhyA and PhyB) (Casal & Sánchez, 1998; Fenner, 2000). The presence of these photoreceptors in seeds serves the purpose of signalling the embryo for dormancy and germination (Footitt et al., 2016) and to work as a signalling layer to promote or inhibit germination through inducing specific enzymes or chemicals (Comrie, 1926). Light also had an effect on seed germination in selected African leafy vegetables (Motsa et al., 2015).

**Plants response to light (Photoreceptors)**

Photoreceptor proteins are light-sensitive proteins involved in the sensing and response to light in a variety of organisms (van der Horst & Hellingwerf, 2004). Five phytochromes (PhyA through PhyE), two cryptochromes (cry1, cry2), and phototropin have been identified in the model species *Arabidopsis thaliana* (Briggs & Olney, 2001; Casal, 2000; Fearon, 2014). Plants photoreceptors include:

**UVR8**

A specific gene in plants called “Chalcone synthase CHS” has been found to respond to UV light and most recently research discovery of UV-B photoreceptor protein in plants called UV resistance locus 8 (UVR8) responds to the wavelength of 280-315 nm (Jenkins, 2014). The UVR8 has a feature that helps it to utilize UV-B through tryptophan unlike other types of plants photoreceptors protein, which uses a
chain of the chromophore (Christie et al., 2012). Short wavelengths of UV, especially UV-B, have high energy and can damage DNA in plants leading to mutations.

**Cryptochromes and Phototropins**

Cryptochromes are photoreceptors arbitrating light management of vegetation in plants (Lin & Shalitin, 2003). Including phycoerythrin, a red pigment, and phycocyanin, a blue pigment, both found in seaweeds. Phototropin is a protein responding to blue light has been distinguished as a chromoprotein arbitrating phototropism. Phy3 is genetically similar to phytochrome and phototropin. Phototropin genetic pattern has identified, and more investigation and research is still ongoing to find more properties of this photoreceptor and its function in plant development. Although it was established that phototropism, chloroplast relocation, and stomatal conductance arbitrated by phototropin; phot1 and phot2 in response to blue light in *Arabidopsis thaliana*, yet the mechanism for this arbitrating differs than other plants species (Briggs & Olney, 2001; Harada, Sakai & Okada, 2003; Wada, Kagawa & Sato, 2003).

The blue region (450-495 nm) in visible light spectrum lays between violet (380-450nm) and green (495-570nm) light (Bruno & Svoronis, 2005). Blue light has high energy and is an influential driver of plant growth and development. All types of light radiation, including blue light, consist of photons or packets of energy (quanta) and the photons in the blue spectrum are known to be absorbed by pigments which pass on the energy to drive the process of photosynthesis. Pigments have limited energy level intake and despite the fact that blue light photons have higher energy than green, red, and other wavebands of light (Blue = 4.4*10^-19 J, Red = 2.8*10^-19 J) (Tennessee, 2019), it is assumed that blue light is less effective in driving photosynthesis due to wasted energy during the energisation of the blue pigments. Nevertheless, blue light can be used as an individual radiation source for indoor plant growth due to its efficiency at a low light intensity (Takemiya et al., 2005). Furthermore, blue light is known to be signalling some of the somatic functions of plants. In general, using monochromatic blue wavelengths on plants result in shorter, smaller and thicker plants with dark green leaves in comparison with plants grown in the absence of blue light or in combination with red light (Runkle, 2007).
**Phytochromes**

This photoreceptor is associated with the growth of plants in response to red and far-red light (Butler, Lane & Siegelman, 1963). It is found in two interconvertible states, \( P_r \) related to red light 650-670 nm and \( P_{fr} \) reacting to far-red light 705-740 nm. Studies have shown that PIF3 reacts with phytochrome, in this case, far-red light acts as a switch between \( P_{fr} \) and \( P_r \) state. The studies also have shown that, depending on the light, phytochromes have a phosphorylating function towards (PKS1) protein and confirming the proposal that phytochrome B has a passive regulating role (Ni, Tepperman & Quail, 1999; Rüdiger et al., 1983). The red range of visible light has a wavelength of 620-750 nm. Studies showed that treating various plant species with monochromatic red light increased the fresh-dry weight, number of leaves, number of branches and stem width (Poudel, Kataoka & Mochioka, 2008). These parameters are all associated with growth rate, leaf net photosynthetic rate, and leaf lifespan. However, these attributes tend to be restricted to plant species with flatter leaf surfaces (Shipley & Vu, 2002).

Far-red is a range of light with a wavelength between 710-850 nm. Far-red is absorbed by plants as a consequence of the absorbing function of chlorophyll for the rest of the light spectrum. It is detected in the plant by phytochrome pigments (Pettai et al., 2005). Studies have recently shown the importance of far-red light by the increase of the efficiency of photosynthesis in reaction to the addition of far-red light to a combination of red/blue LED light with lettuce plants (van Iersel et al., 2016).

UV is part of the electromagnetic spectrum that has a range of wavelength from 10 nm to 400 nm and has high energy sufficient to cause DNA damage in both plants and animals. The very low wavelength UV with the highest energy (UVC) in solar light is filtered out by the ozone layer in the atmosphere, but UVB and UVA are present in solar light reaching the earth’s surface. Most plants have to protect themselves from the damaging effects of UV light by absorbing the energy and dissipating it into complex biochemical compounds. UV energy is too high to be captured by photosynthetic pigments. The quality of phytochemical components of medicinal plants can differ by light exposure and in one of the cases, treating Feverfew with UV A-B increased the parthenolides levels but decreased levels of phenolic acid (Fonseca et al., 2006).
The wavelength range between 495–570 nm of visible light is known as the green light. The green pigment chlorophyll (a key element in photosynthesis) is responsible for the green appearance of plants. This is due to higher absorption of blue and red wavelengths of light more than the green range of light and this results that human eye sees most plants as green (Nishio, 2000).

**Figure 2: Photoreceptors of plants (Ouzounis, Rosenqvist & Ottosen, 2015). Figure 2 has been removed due to Copyright restrictions.**

**History of LEDs**

Light emission by semiconductor was really reported by the British engineer and inventor H J Round in 1907. LEDs were developed when Oleg Losev, in 1927, hypothesised the mechanism of light emission, but the basics of the invention of LEDs was not investigated until 1955 when LEDs were demonstrated as a light energy source that can be monitored in the form of emitted infrared radiation (Braunstein, 1955). The first practical visible light-emitting diode was devised in John Allen's group at the Services Electronics Research Laboratory. A small production line was set up there in 1962. The material used was gallium phosphide containing controlled amounts of zinc and oxygen. They called these devices "crystal lamps"; the term "light emitting diode" came later. Gallium phosphide: zinc, oxygen devices dominated the LED market until the late 1970s (Monemar, Kittler & Grimmeiss, 2008). A patent for LEDs was published in 1962 (Biard & Pittman, 1966) when it was found out by accident that when an electric current passes through a semiconductor made of gallium arsenide GaAs, the result is the
emission of infrared radiation. Using a gallium arsenide phosphide (GaAsP) diode, Nick Holonyak Jr. presented, in 1962, the first prototype LEDs that emitted visible red light (Holonyak Jr & Bevacqua, 1962). In 1972 a yellow, red and red-orange LED blueprint was published based on the same GaAsP principle of Holonyak’s LED by (Craford et al., 1972). Later in 1994 (Nakamura, Mukai & Senoh, 1994) developed more practical forms of LEDs emitting a brighter light. In 1994 (Ignatius & Martin, 1994) demonstrated the first apparatus which included LEDs used as an artificial source of radiation for plant growth. Different types of semiconductor diodes where then developed according to the Holonyak LED model to generate different types of light wavelength for agriculture (Gupta, 2017). Since then LEDs considered an important element in any plant production unit. A fully closed, insulated, multi-tier hydroponic growing facility supplied with temperature, humidity, PH, and EC monitoring systems, installed with interchangeable LED light units is commonly called Plant Factory. The lighting element in most early indoor vertical hydroponic systems consisted mostly of Fluorescent lamps, but LEDs have now become the focus of many researchers and agricultural industries due to their smaller size, efficient energy utilization in generating light and overall energy saving. Although there are many advantages of plant factory systems, they are not a substitution for other agricultural practices. Instead, the expansion of interest in plant factory systems has generated new marketing possibilities with the aid of LEDs. Japan, United States, United Kingdom and more countries from Europe and the Middle East (GrowGroup I. F. S., 2018) has adopted plant factory systems for commercialized agricultural productions (Kozai, 2013b).

Basil

The etymology of Basil is derivative from Greek “Basilikon” which believed to mean “Royal plant” or “King’s plant” deduce from the use of the herb in royal fragrance production. Ocimum basilicum L. (Basil) is a genus in the Labiatae family and grows wild in tropical and sub-tropical climates and known to grow in all populated areas around the world (Dictionary, 2019; Makri & Kintzios, 2008). Basil (Ocimum sp. L.) considered an important culinary herb used for human consumption. Fresh Basil unique taste and flavour made it an essential element in many culinary forms and techniques; pickling, in
flavouring oils, as relishing for sauces/stews and in salads. Pizza is a wide world food item globally spread over 50 countries with a global market expected to reach 233.26 billion dollars in the next five years according to Global Pizza Market Report. Basil is one of the main elements in the making of a pizza, which shows the wide-scale demand of Basil in the food industry. Furthermore, in California, the United States, almost 400 restaurants used Basil daily in their items either as a main flavoured element or as a garnish. Fresh Basil (Ocimum basilicum) is more preferable for consumption due to their large leaves and strong aroma. The food industry does not only used fresh Basil but also using dried Basil by manufactural methods to provide it for consumption or favouring liqueur. The next step of dry Basil usage is the production of essential oils. The essential oils of Basil used widely in baking, confectionery industry, liqueurs, fragrances, and cleaning products (Abbasi et al.; Helstosky, 2008; Keegan, Green & Fu, 2005; Ltd & Markets, 2019; Putievsky & Galambosi, 1999).

Basil still been used as a traditional panacea and in the medical products industry and has reported medicating; renal system problems, abdominal pains, mild nervous behaviours, rise in body temperature, insect bites, nausea, inflammation, migraines, respiratory tract infection symptoms, skin problems. Dentistry used biochemicals derived from Basil in oral hygiene production. Moreover, In Puerto Rico, North America, Sweet Basil used as flatus-relieving medicine for gastrointestinal disorders and found to increase milk production in nursing mothers (Dasgupta, Rao & Yadava, 2004; Holm, 1999; Kathirvel & Ravi, 2012; Lee et al., 2005; Murugan, Murugan & Noortheen, 2007; Simon, Quinn & Murray, 1990; Vieira & Simon, 2000). Essential oils of Basil found to have antioxidant activity (Lee et al., 2005). Studies have shown that these activates work through the inhabitation of oxidization chain reaction which results in destructive effects in plants cellular levels (Ebisch et al., 2006; Studer, Craven & DeRubertis, 1997). Nonclinical studies demonstrated that biochemicals of Basil such as Eugenol (C10H12O2) could help to hinder carcinoma of epidermis, liver and respiratory with the function of antioxidants in high levels, genetic mutation, necrobiosis, and by impeding on to genesis and/or secondary malignant growths. Basil biochemicals found also to retard the growth of cancer cells. Those biochemicals, including an abundance of Linalool (C10H18O) and Eugenol (C10H12O2), found to help
hinder the spread cancer cells. Solvent extraction of Basil containing phytochemicals such as Eugenol (C10H12O2) with the consecration of 0.36 % found to have antineoplastic properties. Basil extraction, containing Eugenol, reported to have a positive effect on programmed cell death in Carcinoma and additionally had a hindering effect of cancer cell growth. Sweet Basil essential oils, containing methyl cinnamate (C10H10O2), Linalool (C10H18O), found to have a toxic effect on the cells of cervical cancer, carcinoma, and mouse embryonic fibroblasts (Baliga et al., 2013; Kathirvel & Ravi, 2012; Magesh et al., 2009; Shimizu et al., 2013).

The trend in growing population consequently increased exigency for all type of resources. Aromatic plant species are considerably demanded due to their collinearly, industrially and medically importance. Hence intensification the need to come up with the ultimate Basil growing recipe. The volatile oils of Basil affected by cultivation, climate conditions, development stages, and the genotypic differences between cultivars in chemical composition and more (Makri & Kintzios, 2008). According to the literature, there is a diversity of procedure for obtaining the essential oils of Sweet Basil (Cassel et al., 2009; Charles & Simon, 1990; Lucchesi, Chemat & Smadja, 2004).

The herb in question used widely in industrial production of important chemicals. A chemical analysis of volatile oils of Basil showed the presence of 29 biochemicals when the volatile oil was prepared by the hydro-distilled method. The presence of those biochemicals affected by growing conditions in the open-filed environment. Using the same extraction method, 25 biochemicals identified from wild-grown plants. A hydro-distilled extraction of market bought Sweet Basil resulted in essential oil with only 19 bio-component. Accumulative record of the composition of the essential oil of Sweet Basil reported the presence of over 60 components depending on the different methods of cultivation, extraction, and quality. A chemotypes analysis showed the mutability of chemo-taxonomical diversity of Sweet Basil (Carvalho et al., 2016; El-Soud et al., 2015; Hussain et al., 2008; Joshi, 2014; Krüger, Wetzel & Zeiger, 2002).
The present study has chosen the five most common components of the essential oil of Sweet Basil appearing in the literature in the literature;

**Estragole (C10H12O);** known as Estragon, Chavicol methyl ether, Methylchavicol, Chavicol methyl ether, and/or Isoanethole. Estragole is a phenylpropene, with a boiling point of 215-216 °C, found naturally in Tarragon, Sweet Fennel, and Anise additionally to Basil. Wild Sweet Basil flowers hydro-distilled extraction can result in the highest concentration of Estragole more the leaves and stems essential oil. Estragole used in perfume manufacturing and as a food additive for flavour. It has found insecticide properties on fruit flies, reaching toxic levels in 8-38 minutes of exposure. However, the response to the Estragole differs from one species of fruit fly to another. Furthermore, Estragole is an effective insecticide agent; Rice weevil, Legume Beetles and Tobacco Beetle. Notwithstanding, it was claimed that Estragole had a carcinogenic effects on mice and require more studies on the effect of long term exposure (Avetisyan et al., 2017; Chalchat & Özcan, 2008; De Vincenzi et al., 2000; Gonzalez-Coloma et al., 2013; Ling Chang, Kyu Cho & Li, 2009; Sigma, 2019b; Smith et al., 2002; Williams, 1997). In the current study (Figure 24), the growth conditions have promoted the accumulation of Estragole although the lighting conditions did have no much effect on the relative abundance of the biochemical.

**Linalool (C10H18O);** also named (3, 7-Dimethyl-1, 6-octadien-3-ol), (3, 7-Dimethyl-3-hydroxy-1, 6-octadiene). It an organic Monoterpenes -terpene alcohol found in more than two hundred plant species, with a boiling point of 197.5°C, which used widely as a scent in many hygiene products and cleaning agents. Linalool can be used through fumigation against American Wheat Weevil, as antifeedant to Aphids, as a toxic fumigant to Melon Thrips or as a repellent against Gall Midge Flies. The antibiotic properties of Basil’s essential oils reported and contributed to the presence of Linalool alongside with the taste of Basil. The light quality found to have an impact on Linalool. Ultra-Violet-B light reported to inhabitation effects on the accumulation of Linalool in young Sweet Basil but had a great effect on older plants. This indicates the importance of the effect of the interaction between development stages and light quality. Different coloured mulches had a significant effect on the accumulation on Linalool.
of fresh Sweet Basil extraction when Red and Blue coloured mulches promoted the lowest accumulation of essential oil, Linalool, respectively (Bagamboula, Uyttendaele & Debevere, 2004; Gonzalez-Coloma et al., 2013; Johnson et al., 1999; Loughrin & Kasperbauer, 2003; PubChem, 2019a; Simon, Quinn & Murray, 1990; Wallace et al., 1991). In the current study, a combination of Blue light (459 nm) and Red light (632 nm) promoted the high accumulation of Linalool in dry Sweet Basil’s essential oil compared with other lighting conditions in a controlled environment (Figure 24). Linalool presence was the highest among the rest of the biochemicals chosen for this analysis.

**Eugenol (C10H12O2)** had many synonyms; (4-Allyl-2-methoxy phenol), (2-Methoxy-4-(2-propenyl) phenol), (Eugenic acid), (Caryophyllic acid), (1-Allyl-3-methoxy-4-hydroxybenzene), (Allylguaiacol), (2-Methoxy-4-allylphenol) and (4-Allyl catechol-2-methyl ether). Eugenol had a boiling point of 252 °C and considered the main component in the essential oil of Cloves, Cinnamon, Bay, and Basil. The aroma of those plant species related to the presence of Eugenol and Chavicol. Eugenol found to have a hindering effect on the development of heterotrophic, Gram-negative, rod-shaped bacteria mainly found in semitropical areas growing on meat and poultry, which give Eugenol the advantage of safe usage in food preserving. Furthermore, Eugenol found to have antibiotic, antimitotic, and anti-inflammatory properties. It reported to have repellent properties against castor bean tick and other insects and considered environmentally unassailable. Light, was reported, to have a significant effect on the accumulation of Eugenol in Sweet Basil when a combination of Red, Blue and Green LED arrays promoted the highest accumulation of Eugenol compared with natural-greenhouse light conditions. Ultra-violet-B light had a significant effect on the accumulation of Eugenol but only in developed plants rather than young ones, which showed the lowest accumulation. On the other hand, Red and Blue coloured mulches inhibited the accumulation of Eugenol in Sweet Basil compared with Black, Green, Yellow mulches. This indicates the effects of light on the accumulation of biochemicals (Abaul, Bourgeois & Bessiere, 1995; Bagamboula, Uyttendaele & Debevere, 2004; Bhuiyan, Begum & Akter, 2010; Gonzalez-Coloma et al., 2013; Johnson et al., 1999; Loughrin & Kasperbauer, 2003; Mallavarapu et al., 1995; Sigma, 2019d; Simon, Quinn & Murray, 1990). In the current study, an amalgamation of
Blue LED light peaked of 459 nm and Red LED light peaked at 632 nm resulted in the highest presence of Eugenol ($P < 0.001$) in Sweet Basil when compared with White LED and HPS lighting in a controlled environment (Figure 24).

**E Cinnamate** ($C_{11}H_{12}O_2$) known as (Ethyl Cinnamate), (3-Phenyl-2-propenoic acid ethyl ester) and/or (Ethyl 3-phenyl-2-propenoate) is the resulted ester from the reaction of Cinnamic acid with ethyl. It has a boiling point of 271 °C. Basil, Wormwood, and Galangal considered some of the natural sources of E Cinnamate in nature. Ethyl Cinnamate reported having a possible widening effect on blood vessels, which would result in reducing high blood pressure, muscular convulsion, and respiratory disorder. E Cinnamate has a positive effect on treating fungal skin infections, moulds, and yeasts. Ethyl Cinnamate usually extracted from plant species material using a hydro-distilled method. Due to its unique aroma, it is used by cosmetologists in making of beauty and cleaning products. E Cinnamate validated by the American Federal agency of Food and Drug Administration (FDA) as a flavouring agent. Due to its multiple benefits, it is found to be used annually around 10 metric tonnes. E Cinnamate accumulation, alongside with other components of the essential oil of Basil, was reported to be influential by development stage and cultivation adjustments (Bhatia *et al.*, 2007; Dubey *et al.*, 2000; Othman *et al.*, 2006; PubChem, 2019b; Ravid *et al.*, 1992; Sigma, 2019c; Sims *et al.*, 2014; Telci *et al.*, 2006). The results in the current research demonstrated the capability of enhancing the accumulation of Ethyl Cinnamate in enclosure farming by using artificial lighting with specific spectrum characteristic. The accumulation of E Cinnamate was significantly higher ($P < 0.001$) when Sweet Basil was cultivated under a mix of Red and Blue LEDs in comparison with White LEDs and HPS lamps (Figure 24).

**Eucalyptol** (C$_{10}$H$_{18}$O) called 1,8-Cineole or 1,8-Epoxy-p-menthane is a Monoterpenes, with a boiling point of 176-177 °C, named for the first time in the mid-nineteenth century by means of steam distillation. The increasing interest in Eucalyptus oil paved the path for the development of essential oil extraction for other plant species. Later the innovation of Gas Chromatography, Mas, and Infrared Spectrometry increased the knowledge about the specific content of those essential oils. Eucalyptol extracted from Sweet Basil reported having antifungal properties. Oils rich with Eucalyptol used widely
in medical production, food flavouring, and aromatic element in cosmetology and as an insect repellent. The main provider of Eucalyptol is the genus Eucalyptus hence the name of the chemical compound. Basil found to accumulate Eucalyptol in combination with other biochemicals in the obtained essential oil. This accumulation possibly regulated by cultivation conditions when Nitrogen and Sulphur found to have a positive impact on the concentration of Eucalyptol. Furthermore, the location of greenhouses used to cultivate Sweet Basil for the extraction of Eucalyptol found to be affecting the accumulation rate. Eucalyptol affected by the development stage as well where the flowering stage promoted the lower accumulation in Sweet Basil leaves. Light has a direct effect on the concentration of Eucalyptol in the essential oil of Sweet Basil when light quality at May and September in Tennessee, United States, resulted in highest Eucalyptol accumulation using supplemental LED lighting compared with the rest of the year. A combination of Red LED light wavelength peaked at 627 nm with Blue LEDs peaked at 447 nm promoted the accumulation of Eucalyptol in hydroponically cultivated Sweet Basil. This accumulation had variations depending on the time of the year is was established and depended on the Red/Blue ratio when a ratio of 1/1.5 Red to Blue gave the highest Eucalyptol concentration in the volatile oil of Sweet Basil compared with the other eight ratios of lighting. Additionally, a 24 hours LED supplementary lighting provided high concentration of Eucalyptol compared with 3, 6, 9, 12, 18 hours exposure (Boland, Brophy & House, 1991; Hammock, 2018; Nurzyńska-Wierdak, 2012; Oxenham, Svoboda & Walters, 2005; Sigma, 2019a; Zheljazkov et al., 2008). Applying Red and Blue LED as a full growth lighting for Sweet Basil resulted in highest Eucalyptol abundance ($P < 0.01$) when compared with White LED and HPS lighting as the results in the present analysis shows (Figure 24).

**Basil and light**

Plants grow by photosynthesis but only a small amount of the radiation in solar energy is used in photosynthesis (Ort, Zhu & Melis, 2011). Additionally, light spectra and intensity control the concentration of biochemical compounds in many leafy vegetables. LEDs can help establish the basic roles for light sources that help to enhance commercial plant production. Furthermore, LEDs are considered the most suitable method to investigate and understand the effects of light on the
physiological and biochemical characteristics of plants (Veitch, Lada & MacDonald, 2012). Plant growth depends mainly on the blue and red light yet numerous researchers, as shown in (Table 1), demonstrated contrasting findings stating in general that treating many plant species with different types of lights such as UVA-B, far-red or green integrated with other LEDs, enhanced the growth, yield, and nourishment value of those plants. Furthermore, according to (Lim & Eom, 2013) treating sweet Basil with monochromatic blue LEDs (460 nm) was most effective at improving root formation and consequently growth and yield. Root establishment was about three times faster upon treatment with blue light than natural sunlight but induced dark green leaves which contradicted the findings of (Runkle, 2007).

According to Amaki different light conditions affected the growth of Sweet Basil. Seedlings treated with green LEDs 525 nm grew rapidly. Green LEDs gave the largest fresh weight of the whole plant, fresh weight of leaves, stem diameters, and root growth, while lateral shoots were nurtured under blue LEDs 470 nm. Amaki also indicated that using blue-green LEDs would result in sub-standard growth of roots and thus poor growth rate for the whole plant. The leaf surface bent outward and often downward under red LEDs 660nm and stems were thinner when grown under monochromatic red LEDs. The root growth rate of Sweet Basil (Ocimum basilicum) was promoted using blue LEDs 460 nm three times faster than natural sunlight (Lim & Eom, 2013). Exposing the plants, to blue LEDs for longer periods led to the etiolation of older plant leaves. Treating Basil plants with a combination of high red with a low amount of red: far-red LEDs resulted in higher growth rate and greater total biomass while treating plants with a combination of high blue, high green, red: far-red and 1% UV LEDs resulted in the greatest total biomass, best root: shoot ratio (R/S) and higher total phenolic content. High red: far-red ratio LEDs resulted in the longer root system and faster development than other treatments (Bantis, Ouzounis & Radoglou, 2016).

Contrary research on effects of light on sweet Basil (Ocimum basilicum L.) demonstrated that using a different combination of red, blue, green and UV-A LEDs had no effect on fresh and dry weight, leaf area and leaf fresh weight or plant height of Basil when compared to monochromatic LEDs. The research
indicated the importance of blue light 447 nm to promote thicker Basil leaves whilst green light 527nm seem to thin them (Jensen, Clausen & Kjaer, 2018).

Plants produce energy (Glucose) by performing photosynthesis. This energy is vital for the plants to grow. Maximum photosynthesis ($A_{max}$) will consequently lead to maximum plant yields when other environmental conditions are fixed (Fischer et al., 1998). The light quantity has an important effect as on $A_{max}$ of plants as shown by Bowes, Ogren & Hageman (1972) when an increase in light intensity up to (400 $\mu$mol m$^{-2}$ s$^{-1}$) resulted in an increase in $A_{max}$ and in light saturation for soybeans and Basil. A light intensity of 70 $\mu$mol m$^{-2}$ s$^{-1}$ supplied by Blue (440 nm peak) and Red (650 nm peak) LEDs resulted in an increase in $A_{max}$ to values compared with other light treatments as shown by (Kim et al., 2004b).

The effects of two light intensities (200 and 500 $\mu$mol m$^{-2}$ s$^{-1}$) on tomato, cucumber, pepper, soybean, lettuce and wheat had a negative interaction between low light intensity and the increase in Blue light (400-500 nm) but there was no indication of the specific wavelength of Blue used for all plant species (Snowden, 2015). This interaction affected the DW and LA of the plants negatively at a high light intensity which can be explained as plants go through stress under high intensity rather than the effect of Blue light as illustrated by (Quiles, 2006) when high light intensity inhibited the photosynthesis process of oat leaves. On the other hand, high light intensity (400 $\mu$mol m$^{-2}$ s$^{-1}$) increased arachidonic acid in Fuji cherry (*Prunus incisa*) (Solovchenko et al., 2008). There was a positive impact as well of high light intensity (90 W.m$^{-2}$ $\approx$ 20,000 lux $\approx$ 282 $\mu$mol m$^{-2}$ s$^{-1}$) on Radish plants (Lichtenthaler et al., 1981). This indicated the different light intensity requirements for each plant species. The results shown in this study showed the positive impact of using high light intensity (470 $\pm$ 20 $\mu$mol m$^{-2}$ s$^{-1}$) in the cultivation of Sweet Basil as a proximate mimic to the natural conditions for *Ocimum* growth that thrives in hot-humid conditions in parts of Africa, Asia, and America (Hiltunen & Holm, 1999). In those areas, the light intensity might reach up to 1600 $\mu$mol m$^{-2}$ s$^{-1}$ as measured near El Verde Field Station, Puerto Rico in North America (de Castro, 2000). For example; A light intensity of 282 $\mu$mol m$^{-2}$ s$^{-1}$ would promote higher DW than plants grown under 14.1 $\mu$mol m$^{-2}$ s$^{-1}$ but increasing the light intensity up to
365 μmol m$^{-2}$ s$^{-1}$ resulted in higher value of DW than plant treated with 255.5, 146, 29.2 μmol m$^{-2}$ s$^{-1}$ respectively (Friend, Helson & Fisher, 1962; Lichtenthaler et al., 1981).

The contrasting findings of the effects of different light conditions on plant growth, development, yield, and quality leads to the conclusion that further investigation on the effect of light quality on the growth and quality of Basil is required.
Table 1: Summary of the literature

<table>
<thead>
<tr>
<th>AUTHOR</th>
<th>PLANT SP.</th>
<th>TREATMENT</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Shiga et al., 2009)</td>
<td>Sweet Basil (<em>Ocimum basilicum</em> L.)</td>
<td>W (normalized at 613 nm)</td>
<td>Both antioxidant activity and phenolic content were increased.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R 600-700 nm (normalized at 660 nm)</td>
<td>Increase in Rosmarinic acid</td>
</tr>
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<td></td>
<td></td>
<td>B 400-500 nm (normalized at 451 nm)</td>
<td>Level of Rosmarinic acid was only 3 mg g⁻¹</td>
</tr>
<tr>
<td>(Chang, Alderson &amp; Wright, 2008)</td>
<td>Basil cv. ‘Basil Sweet Genovese’</td>
<td>No shade with 24.9 moles m⁻² d⁻¹ light integrals.</td>
<td>No difference in CO₂ assimilation rate</td>
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<td></td>
<td></td>
<td></td>
<td>Reduced leaf temperature</td>
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<td></td>
<td></td>
<td></td>
<td>Linalool and eugenol increased</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>No differences in the relative content of 1,8cineole</td>
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<tr>
<td></td>
<td></td>
<td>25% glasshouse irradiance with 13.5 moles m⁻² d⁻¹ light integrals.</td>
<td>No difference in CO₂ assimilation rate</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Reduced leaf temperature</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No differences in the relative content of 1,8cineole</td>
</tr>
<tr>
<td>Irradiance Level</td>
<td>Treatment</td>
<td>Observations</td>
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<tr>
<td>-----------------</td>
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<tr>
<td>50% glasshouse irradiance.</td>
<td>Methyleugenol was increased No differences in the relative content of 1,8-cineole</td>
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<tr>
<td>75% glasshouse irradiance WTH light integral of 5.3 molesm(^{-2}) d(^{-1}).</td>
<td>Shorter plants, lower weight, smaller leaf area, fewer shots and higher specific leaf area, and also strongly reduced the rate of photosynthesis Leaf temperature increased Strongly reduced the total volatile oil content in fresh leaves, especially in older plants. No differences in the relative content of 1, 8cineole</td>
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</table>

(Loughrin & Kasperbauer, 2001) Basil (*Ocimum basilicum* L.)

<table>
<thead>
<tr>
<th>Row Covers</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>R polyethene row covers at 645 ± 5 nm: FR 735 ± 5 nm</td>
<td>Leaves have a greater area, succulence and fresh weight</td>
</tr>
<tr>
<td>B polyethene row covers</td>
<td>Low levels of (\beta)-pinene, myrcene, 1, 8-cineole, camphor, linalool, terpinen-4-ol, bornyl acetate, (\alpha)-terpineol, hexanal, octyl acetate, eugenol Less amount of DW</td>
</tr>
<tr>
<td>W polyethene row covers</td>
<td>Higher concentrations of aroma compounds Leaves have higher concentrations of phenolics.</td>
</tr>
<tr>
<td>(Johnson et al., 1999)</td>
<td>Sweet Basil (<em>Ocimum basilicum</em> L.)</td>
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<tr>
<td>(Jensen, Clausen &amp; Kjaer, 2018)</td>
<td>Sweet Basil (<em>Ocimum basilicum</em> L.)</td>
</tr>
</tbody>
</table>

Low levels of α and β-pinene, myrcene, 1, 8-cineole, camphor, linalool, terpinen-4-ol, bornyl acetate, α-terpineol, hexanal, octyl acetate, eugenol

G polyethene row covers

Higher concentrations of aroma compounds

Leaves have higher concentrations of phenolics
The content of soluble sugars and the distribution of individual fatty acids generally showed no significant differences.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Results</th>
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</thead>
<tbody>
<tr>
<td>80%R/20%B +UV-A</td>
<td>No differences in production yield: FW, DW, leaf area or height.</td>
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<tr>
<td></td>
<td>No effects on the leaf DW to stem DW ratio was observed.</td>
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<tr>
<td></td>
<td>Highest shelf-life performance.</td>
</tr>
<tr>
<td></td>
<td>The content of soluble sugars and the distribution of individual fatty acids generally showed no significant differences.</td>
</tr>
<tr>
<td>40%R/60%B</td>
<td>Leaf mass per leaf area was significantly lower.</td>
</tr>
<tr>
<td></td>
<td>No differences in production yield in FW, DW, leaf area or height.</td>
</tr>
<tr>
<td></td>
<td>No effects on the leaf DW to stem DW ratio was observed.</td>
</tr>
<tr>
<td>Ratio</td>
<td>Effect</td>
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<td>---------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Increasing ratios of blue light stimulate leaf thickening.</td>
<td></td>
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<tr>
<td>Higher abaxial SD.</td>
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<tr>
<td>Higher levels of abscisic acid and abscisic acid glucosylester.</td>
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<tr>
<td>The content of soluble sugars and the distribution of individual fatty acids generally showed no significant differences.</td>
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</tr>
<tr>
<td>80%R/20%G</td>
<td>No differences in production yield in FW, DW, leaf area or height.</td>
</tr>
<tr>
<td>No effects on the leaf DW to stem DW ratio was observed.</td>
<td></td>
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<tr>
<td>Decrease stimulates leaf thickening with an increase in G.</td>
<td></td>
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<tr>
<td>Lowest extent of chilling induced leaf injury, and thus highest shelf-life performance.</td>
<td></td>
</tr>
</tbody>
</table>
The content of soluble sugars and the distribution of individual fatty acids generally showed no significant differences.

(Ahlman, Bånkestad & Wik, 2017)

<table>
<thead>
<tr>
<th>Basil</th>
<th>Basil</th>
<th>Experiment (A): 420, 450, 530 and 630 nm were used at a time.</th>
<th>Lowest light intensities the derivative is highest when changing LED 630, followed by 420, 450 and then 530, for both photosynthetic rate and chlorophyll fluorescence. Photosynthetic yield indicates that up to about 150 µmol m$^{-2}$ s$^{-1}$ it is most efficient to provide light from LED 630 and thereafter one should increase the light from LED 450.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ocimum basilicum ‘Nufar’)</td>
<td>(Ocimum basilicum ‘Genovese’)</td>
<td>Experiment (B): B: R ratio 1:3 and B: R-ratio 3:1 420, 450, 530 and 630 nm were used at a time.</td>
<td>The fluorescence gains were lower for blue intense background light. Changing cultivar and adding background light did not change the order of the derivatives.</td>
</tr>
<tr>
<td>(Loughrin &amp; Kasperbauer, 2003)</td>
<td>Sweet Basil (Ocimum basilicum L.)</td>
<td>Experiment (C): 5 spectra R: 3 spectra B, 40 µmol m$^{-2}$ s$^{-1}$</td>
<td>The fluorescence gains do not continue to increase for the highest light intensities. The gain remains highest for LED 630.</td>
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<tr>
<td>(Carstensen et al., 2016)</td>
<td>Ocimum basilicum (Sweet Basil cv. Nufar)</td>
<td>F-R coloured mulch. R coloured mulch. B coloured mulch.</td>
<td>approximately 50-fold higher than the yield of volatile compounds that found for air-dried Basil leaves</td>
</tr>
<tr>
<td>(Mosadegh et al., 2018)</td>
<td>Basil (Ocimum basilicum L. (cv. Genovese))</td>
<td>UV-B: 8.5 kJ m$^{-2}$day$^{-1}$ UV-B: 34 kJ m$^{-2}$day$^{-1}$ UV-B: 68 kJ m$^{-2}$day$^{-1}$</td>
<td>Phenols content increased in all the doses tested after 48, 72 h Significant phenol content enhancement the dissipation of energy per cross-section drastically dropped after 72 h.</td>
</tr>
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<td></td>
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<td>Phase I: 110 µmol m$^{-2}$ s$^{-1}$ for 2.5 h. Phase II: 530 µmol m$^{-2}$ s$^{-1}$ for 1 h. Phase III: 1750 µmol m$^{-2}$ s$^{-1}$ for 2 h. Phase IV: 110 µmol m$^{-2}$ s$^{-1}$ for 3–6 h.</td>
<td>The capacity to use a certain light intensity is reflected by how fast and how complex the dynamics are. In particular, the results show that optimal model order is a potential indicator of light tolerance in plants that could be a valuable feedback signal for lighting control in greenhouses.</td>
</tr>
<tr>
<td>(Lim &amp; Eom, 2013)</td>
<td>Sweet Basil (<em>Ocimum basilicum</em> L.)</td>
<td>UV-B: 102 kJ m(^{-2})day(^{-1})</td>
<td>Heights phenols content.</td>
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<td></td>
<td>F</td>
<td>Poor in root growth until 6 weeks.</td>
<td>Chlorophyll content did not differ significantly according to light quality</td>
</tr>
<tr>
<td></td>
<td>R 625 nm</td>
<td>Induced slow increases in root growth until 6 weeks.</td>
<td>Shoot growth was stimulated continuously.</td>
</tr>
<tr>
<td></td>
<td>B 460 nm</td>
<td>The most effective at improving root formation.</td>
<td>Root establishment was about three times faster upon treatment with blue light than natural sunlight.</td>
</tr>
<tr>
<td>(Urbonavičiūtė <em>et al.</em>, 2008)</td>
<td>Basil (<em>Ocimum basilicum</em> L.)</td>
<td>R 640 nm supplementary with: R 662nm + B 455 nm + F-R 735 nm.</td>
<td>The slight increase in monosugar content and traces of sucrose.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No remarkable effect on vitamin C concentration in leaves.</td>
<td>Tenuous 5–10 % increase in nitrate ion concentration was found.</td>
</tr>
<tr>
<td>(Chang, 2005)</td>
<td>Basil</td>
<td>UV-B</td>
<td>R 640 nm</td>
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<tr>
<td>The high flux of red solid-state light stimulated the vital activity of plants.</td>
<td>The slight increase in monosugar content and traces of sucrose. No remarkable effect on vitamin C concentration in leaves. Tenuous 5–10% increase in nitrate ion concentration was found. The high flux of red solid-state light stimulated the vital activity of plants.</td>
<td>Short plants with higher dry matter and thicker leaves. Stimulated the synthesis of volatile oil compounds, i.e. phenyl-propanoids (eugenol) and terpenoids (notably 1, 8-cineole and linalool). There was no effect, however, on volatile oil composition.</td>
<td>Shorter plants with less dry matter and smaller leaves.</td>
</tr>
</tbody>
</table>
The volatile oil content of the leaves was greatly increased. The content of eugenol was decreased whereas the content of β-myrcene was increased. There were no effects on the relative contents of 1, 8-cineole, linalool and other compounds.

(Bantis, Ouzounis & Radoglou, 2016)

<table>
<thead>
<tr>
<th>Treatment Description</th>
<th>Volatile Oil Content</th>
<th>Growth and Biomass</th>
<th>Phenolic Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Lettuce leaf Basil.</td>
<td>High R &amp; R: FR LED.</td>
<td>Longer root system than other treatments and faster root system development.</td>
<td></td>
</tr>
<tr>
<td>(B) Red Rubin-mountain Anthos hybrid Basil.</td>
<td>High R &amp; Low R: FR LED.</td>
<td>Higher growth rate and greater total biomass.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate B, R &amp; Low R: FR LED.</td>
<td>Higher growth rate and greater total biomass.</td>
<td></td>
</tr>
<tr>
<td>Control: FL High B, G &amp; R: FR. PPFD: for (28) days and (14) H PP.</td>
<td>Higher growth rate and less root: shoot ratio (R/S). Greater leaf area of both. For (Lettuce leaf Basil) only, seedlings quickly developed a new root system.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Research Question

LEDs are commonly used for vertical, hydroponic farming systems (plant factory). LEDs have great features over incandescent, fluorescent, and HID lamps. Most of the commercial agricultural LED lighting system manufacturers adopted the McCree curve in their design for the lighting system. However, the McCree curve does not give an accurate representation of individual plant spectral needs. Moreover, there are several scientific gaps in the reported paper by McCree which makes it difficult to generalize the results obtained on all plant species. Therefore, this research will investigate the effect of light quality (wavelength) on the yield and quality of Basil. The project is based on the principle of providing a spectral combination that matches the plant needs. Basil spectrum absorption to be determined using a spectrophotometer and LED light array to be designed with output to match Basil light absorption. This will be the first study the consider Basil light requirements and is believed that this will have a great impact on the growth, yield and quality of Basil. The proposed project could have a great impact in the field of Basil production under both controlled (LED as a sole lighting source) and semi-controlled (LED as a supplementary lighting source).

Recommendations

The literature demonstrated contradiction on the effect of different light conditions on plants on both supplementary and sole lighting LED systems. Furthermore, the findings on the effect of monochromic and combined LEDs are not clear. More and intensive analytic research is needed in both areas to gain a clearer understanding on the precise light requirements of plants.
Chapter 2

Morphological and physiological responses of Sweet Basil (*Ocimum basilicum*) to different types of hydroponic systems and culture media
**Introduction**

Optimization of growing plant species has been and still is the focus of attention of many researchers. This desire transpires from the need for global food sufficiency but aiming at the same time towards the sustainability of resources used in the process. Researchers have established that cultivation of plant species in open-fields has many inefficiencies. One of the most important factors affecting plant growing in open-fields is irrigation. Agricultural, agronomical, and horticultural irrigation systems are considered sustainable only if the enhancement of plant species cultivation is achieved with minimum water waste, which is not an easy task due to major water losses by transpiration, percolation, and evaporation. The current attention to sustainability in plant production resource management, especially irrigation practices sustainability, resulted from the realization of water shortage on a global scale. This shortage is predicted to increase as the result of climate change in the future and so increases the need to address this issue with optimum water management for agricultural practices (Mancosu *et al.*, 2015). In relation to the increased population of the world in some areas, resulting in decreased availability of farmlands for crop production more and more natural habitats and non-arable lands have been exploited in the process of civil and industrial expansion (Kline & Alig, 1999; Plaut, 1980). Traditional, open-field cultivation served as the main method of plant species growing since the first glance of civilization development and still faces numerous challenges such as effective utilization of added nutrients due to the complexity of compost compositions. As a proposed solution for the above challenges and more, soilless cultivation of some plant species was developed and is, in fact, an old practice and was performed widely in ancient Egypt, Mesopotamia and Central America as an effective method of plant production (Raviv, Lieth & Raviv, 2008). A form of soilless cultivation was defined for the first time as Hydroponics by Gericke (1938) and described as "the art and science of crop production in liquid culture media" (Gericke, 1938). Since then hydroponic
cultivation of plant species has interested many researchers not only as an important tool for water conservation in plant growing practices but to increase the possibility to boost plant growth and quality. Soilless cultivation can serve as a template for additional soluble elements that might increase the possibility of maximum yield and efficiency of plant growing. Additional elements in the hydroponic system such as; supplementary lighting, further supplementary elements and adjustments in space utilization (multi-tier hydroponics with increased density) have also been the focus of much research development (Ebisawa et al., 2008; Johnson Sr, 1983; Kaya & Higgs, 2001; Keller et al., 2015; Muro et al., 1997; Nam et al., 2016).

**Aims and objectives**

This analysis designed to gain more understanding of the morphological and physiological responses of Sweet Basil (*Ocimum basilicum*) to different cultivation methods. The current study aimed:

- To investigate the effects of Clay Pebble (CP) growing media, Rockwool (RW) hydroponic media, Coco coir (CC) cultivation media, and compost base growing media (C) on the growth and development of Sweet Basil (*Ocimum basilicum*) in a closed semi-controlled environment. Additionally to identify the optimum growing media among hydroponic mediums (CP, RW and CC).

- To determine the optimum hydroponic system for basil growth and production. Four hydroponic systems were tested: Nutrient Film Technique hydroponic system (NFT), Floating/raft system (R), Ebb-flow hydroponic system (E&F) and compared with compost cultivation (C) as a control.
Materials and Methods

1. Sweet Basil cultivation media analysis

Sweet Basil seeds were obtained from Sutton’s Seeds (Paignton, England, www.suttons.co.uk). Thirty-two seeds were sown in John Innes No.1 young plant compost in June 2018 at a glasshouse in Skardon garden, University of Plymouth. Only one repeat was performed per experiment due to shortage of space and time. Seven days after-sowing, Sweet Basil seedlings were transplanted into four, separate growing mediums. The mediums were; Clay pebble (CP) obtained from Holland hydroponics and horticulture (https://www.hydroponics.co.uk/), Grodan VITAL Rockwool (RW) 1m Slab 150mm x 75mm obtained via (https://www.grodan.com/), Coco Coir obtained from Tidy hydro Plymouth (https://www.tidyhydro.com) and compost-based medium (John Innes No.1). 8 pots were cultured from each medium, pot size: 6 x 8 cm (randomly distributed). Floating/raft hydroponic system was used to test the effect of different medium, using three hydroponic tanks 40 x 70 x 20 cm. The hydroponic tanks were filled with 25 litres of water for each tank, (topped up only once throughout the experiment) and 25 ml of Hydro A/B 1L B’Cuzz Nutrients solution – these are highly concentrated mineral basic plant nutrition solutions, Hydro A is rich in calcium which helps the plant against heat stress. Hydro B contains magnesium which enhances the production of chlorophyll, making it an indispensable building material enabling the plant to grow. Hydro A and B also contain; Nitrogen, Phosphorus, Potassium, Sulphur, Iron, Copper, Manganese, Zinc, Molybdenum, Boron Chloride and Sodium - , for each tank, was added to the body of water for each. The compost treatment (control) contained eight pots (6 x 8 cm) watered when needed. The pH and EC regularly monitored in the hydroponic treatments until harvest day and kept at between pH 6.5 – 6.8 and 1.0 – 1.4 dSm⁻¹ EC by adding the Hydro A/B solution when needed. The compost treatment was fertilized once during the duration of the experiment -but not with Hydro A/B solution due to its compatibility only with hydroponics-
using (100 g) of Gro-Sure Farmyard Manure https://www.gardenhealth.com/ per pot. The experiment was carried out in a glasshouse at Skardon garden, University of Plymouth where the light condition was full daylight (June-September 2018) plus supplementary lighting, using High-Pressure Sodium (HPS) lamps, that programmed to turn on when the ambient lighting becomes lower than 10 µmol m$^{-2}$ s$^{-1}$ (7 p.m. - 7 a.m.). HPS lamps in the greenhouse provided 12 ± 2 µmol m$^{-2}$ s$^{-1}$ supplementary light intensity. The average temperature was 18 ± 2 °C, the humidity was 56 ± 2%, and the thermal screen programmed to cover the glasshouse at 24 °C during daytime and 10 °C during the night. Vents were programmed to open at 22°C during the day and 19°C during the night. Heating was set at 18-22 °C with a humidity threshold of 65%.

**Figure 3; Sweet Basil media analysis layout.**

Light saturated instantaneous Photosynthetic rate ($A_{max}$) was measured using an LCi-SD Highly Portable Ambient Photosynthesis System (ADC BioScientific, Herts). The $A_{max}$ was measured from the upper part of the plants (fully developed leaves) in the middle of the day and measured in µg C/m$^2$/s. Chlorophyll fluorescence rate Fv/Fm was measured using a
Hansatech Pocket PEA meter (Hansatech Ltd, Norfolk, UK) in the upper fully developed leaves but different plant leaves were chosen than those used to measure $A_{\text{max}}$. Stomatal conductance (Gs) was measured using a Delta-T AP4 Leaf Porometer (Delta T Devices, Cambridge UK). The Gs was measured, in centimetre per reciprocal second ($\text{cm s}^{-1}$), from fully developed leaves but different leaves than those chosen for $A_{\text{max}}$ or Fv/Fm. Plant height was measured from the end of the root system to the top of the plants and measured in centimetres (cm) using a ruler. Leaf area LA was measured, in square millimetres ($\text{mm}^2$), using a leaf area image analyser LAI; HITACHI KP-D40 colour digital camera with a lightbox and WinDias 1.5 software from Delta-T Devices Ltd. Fresh weight FW was measured, after removing the root system, using a sensitive Fisher Scientific SG-402 laboratory balance. Dry weight DW was measured, using a sensitive Fisher Scientific SG-402 laboratory balance, after plants were dried at 60° C for 96 h (Saha, Monroe & Day, 2016). Both FW and DW measured in grams (g).

2. **Sweet Basil cultivation method analysis**

Sweet Basil seeds were obtained from Sutton’s Seeds (Paignton, England, www.suttons.co.uk). The Sweet Basil seedlings were established in John Innes No.1 young plant compost. Seedlings were transplanted, after 7 days, into four different growing systems using clay pebble medium; A Nutrient Film Technique (NTF) hydroponic system, a Raft/floating hydroponic system (R), an Ebb and Flow system (EBB) and Compost (C) using 8 pots of young plant compost watered when needed for the treatment. All the growing conditions were fixed for both experiment. All hydroponic systems were supplied with water and air pumps, (E&F) was supplied with a water pump that alternate water flow using a timer; 15 minutes on/off. The seedlings were established as in Sweet Basil cultivation media experiment, as well as, environmental conditions, measured physiological, and morphological parameters.
Results

1. Sweet Basil cultivation media analysis

I. Physiological responses

Light saturated maximum photosynthetic rate $A_{\text{max}}$

There was a significant effect of the growing media on the instantaneous light-saturated maximum photosynthetic rate $A_{\text{max}}$ of Sweet Basil ($P \leq 0.001$). $A_{\text{max}}$ was higher in Sweet Basil

![Figure 5. The effect of mediums; CP, CC, RW and C, on $A_{\text{max}}$ of Sweet Basil. (LSD = 0.7).](image)
grown in hydroponic media compared with those grown in compost. Among hydroponic mediums, Sweet Basil that had grown in Rockwool (RW) gave the highest $A_{\text{max}}$ and Coco coir (CC) growing media showed the lowest $A_{\text{max}}$ (Figure 2).

**Stomatal conductance $G_s$**

There was no significant effect of the cultivation media on the stomatal conductance $G_s$ of Sweet Basil ($P = 0.7$) (Figure 3).

**Chlorophyll Fluorescence Rate $Fv/Fm$**

There was a significant impact of culture media on the Chlorophyll Fluorescence Rate $Fv/Fm$ of Sweet Basil ($P \leq 0.001$). $Fv/Fm$ was higher in Sweet Basil grown in hydroponic media compared with those grown in compost. Among hydroponic mediums, Sweet Basil grown in RW gave the highest $Fv/Fm$ among other growing media and CP growing media showed the lowest $Fv/Fm$ (Figure 4).

![Figure 6. The effect of mediums; CP, CC, RW and C on $G_s$ of Sweet Basil.](image)

![Figure 7. The effect of medium; CP, CC, RW and C on $Fv/Fm$ of Sweet Basil. (LSD = 0.02).](image)
II. Morphological responses

Height H

There was a significant impact of growing media on the height H of Sweet Basil ($P = 0.007$). Sweet Basil was taller when grown in hydroponic media compared with growing it in compost. Among hydroponic mediums, Sweet Basil that has grown in RW gave the tallest plants among other growing media and CP growing media showed the shortest plants (Figure 5).

![Figure 8](image)

Figure 8. The effect of mediums: CP, CC, RW and C, on Sweet Basil height. (LSD = 0.02).

Leaf Area LA

Leaf area of Sweet Basil was affected significantly by growing media ($P = 0.001$). Sweet Basil grown in hydroponic media resulted in a high value of LA compared with growing it in compost. Among hydroponic mediums, Sweet Basil that has grown in RW gave the highest LA and CP growing media showed the lowest LA (Figure 6).

![Figure 9](image)

Figure 9. The effect of mediums: CP, CC, RW, and C on LA of Sweet Basil. (LSD = 332).
Fresh Weight FW

There was no consequential effect of growing media on the FW of Sweet Basil ($P = 0.4$) (Figure 7).

![Figure 10. The effect of mediums: CP, CC, RW, and C on FW of Sweet Basil.](image)

Dry Weight DW

There was a notable effect of the cultivation media on the DW of Sweet Basil ($P = 0.03$). Sweet Basil grown in hydroponic media resulted in a high value of DW compared with growing it in Compost. Among hydroponic mediums, Sweet Basil that has grown in RW gave the highest DW and CP growing media showed the lowest DW. However, there was no significant effect between the hydroponic media (Figure 8).
2. Sweet Basil cultivation method analysis

I. Physiological responses

Light saturated maximum photosynthetic rate $A_{\text{max}}$

There was a significant effect of cultivation systems on instantaneous light-saturated maximum photosynthetic rate $A_{\text{max}}$ of Sweet Basil ($P \leq 0.001$). $A_{\text{max}}$ was higher in Sweet Basil grown in hydroponic systems compared with those grown in compost. Among hydroponic systems, Raft system used to grow Sweet Basil promoted the highest value of $A_{\text{max}}$ and the Ebb and Flow hydroponic system promoted the lowest value for $A_{\text{max}}$ (Figure 8).

![Figure 11. The effect of systems: NFT, R, E&F, and C on $A_{\text{max}}$ of Sweet Basil. (LSD = 1.2).](image)

Stomatal conductance $G_s$
There was a significant effect of growing systems on the Stomatal Conductance $G_s$ ($P \leq 0.001$) for Sweet Basil. $G_s$ was higher in Sweet Basil grown in hydroponic systems compared with those grown in compost. Among hydroponic systems, Raft system (R) used gave the highest value of $G_s$ and the Ebb and Flow (E&F) hydroponic system showed the lowest value for $G_s$ (Figure 9).

**Figure 12. The effect of systems: NFT, R, E&F, and C on $G_s$ of Sweet Basil. (LSD = 0.09).**

**Chlorophyll Fluorescence rate $Fv/Fm$**

There was a significant effect of cultivation systems on the Chlorophyll Fluorescence $Fv/Fm$ ($P = 0.002$) of Sweet Basil. $Fv/Fm$ was higher in Sweet Basil grown in hydroponic systems compared with those grown in compost. Among hydroponic systems, Raft system used to grow

**Figure 13. The effect of systems: NFT, R, E&F, and C on $Fv/Fm$ of Sweet Basil. (LSD = 0.07).**
Sweet Basil promoted the highest value of Fv/Fm compared to other hydroponic systems and the Ebb and Flow (E&F) hydroponic system promoted the lowest value for Fv/Fm (Figure 10).

I. Morphological responses

Height \( H \)

There was a significant effect of cultivation systems on Sweet Basil height \( (P \leq 0.001) \). Sweet Basil was taller when grown in hydroponic systems compared with those grown in compost.

Leaf area \( LA \)

There was a significant effect of cultivation systems on the Leaf Area \( LA \) of Sweet Basil \( (P \leq 0.001) \). Sweet Basil leaves were larger when grown in hydroponic systems compared with those grown in compost. Among hydroponic systems, Raft system (R) used to grow Sweet Basil promoted the highest value of \( LA \) compared to other hydroponic systems and the Ebb and Flow (E&F) hydroponic system promoted the lowest value for \( LA \) (Figure 12).
Fresh weight FW

There was a significant impact of cultivation systems on the Fresh Weight FW of Sweet Basil ($P \leq 0.001$). Sweet Basil had higher FW value when grown in hydroponic systems compared with those grown in compost. Among hydroponic systems, Raft system (R) used to grow Sweet Basil promoted the highest value of FW compared to other hydroponic systems and the Ebb and Flow (E&F) hydroponic system promoted the lowest value for FW (Figure 13).
Dry weight DW

There was a significant effect of cultivation systems on the Dry Weight DW of Sweet Basil ($P \leq 0.001$). Sweet Basil had higher DW when grown in hydroponic systems compared with those grown in compost. Among hydroponic systems, Raft system (R) used to grow Sweet Basil promoted the highest value of DW compared to other hydroponic systems and the Ebb and Flow (E&F) hydroponic system promoted the lowest value for DW (Figure 14).

![Figure 17. The effects of systems: NFT, R, E&F, and C of DW of Sweet Basil. (LSD = 6.3)](image)

Discussion

In general, plant species cultivation in any ambience, taking compost in the count, is hydroponic based cultivation. This is because assimilation of nutrient and other soluble components is impossible without the presence of moisture. The benefits of soilless cultures for plants gives it a great advantage compared to soil culture mainly in efficient use of water and nutrition, but also no chemical weed or pest control products are needed. This does not make hydroponic growing an alternative method of production when soil conditions are suitable and available. The chemical and/or biological reactions between compost elements and plants root system alongside with effect of living organisms in compost is a complex balance that affects the assimilation rate. The balance considered a double-edged sword, in so any increase or decrease in any element can have a positive or negative effect on plant growth as a result. This complex biological/chemical balance becomes more straightforward when
switching to soilless hydroponic cultivation method, eliminating the interactions with most compost elements.

Growth parameters in the current analysis of Sweet Basil were higher in hydroponic cultivation compared to growing it in compost. Concurring with this finding, it was established that growing Sweet Basil in raft hydroponics system increased the quality of the herb more specifically enhancement in vitamin C and E, as well as increased Phenols, Thioccic and Rosmarinic acid levels in comparison with compost cultivation. This means a hydroponic cultivation boost the level of biochemical reactions in plants (Sgherri et al., 2010). Growing Basil in hydroponic cultivation increases growth rate as well as enhanced quality and flavour by almost 50% compared to growing it in compost and this was measured by consumers (Jones Jr, 2016). Basil found to thrive and prosper better when switching from the ebb and flow hydroponic system to the NFT system, which shows the effects of different hydroponic techniques on the growth rate of Basil. However, compost cultivation is more tolerant to inaccuracies than hydroponics, which require accuracy and precise management. Furthermore, growing massive lands using hydroponics can be costly compared with compost cultivation on a large scale. Adding to that, the lack of need to hydroponics in some areas where vast lands available for cultivation with no problems in water availability (Jones Jr, 2016). It established that Sweet Basil and other pharmaceutical plant species could produce in hydroponics commercially. Sweet Basil cultivation in hydroponics showed better irrigation management and less water waste. Hydroponics found to reduce water usage by almost 90% compared to compost cultivation of plant species (Sardare & Admane, 2013).

The raft or floating hydroponic technique patented at the United State in 1977 and used lettuce as a subject of analysis. The system suggested in order to optimise the plant growth and increase yield by seven folds compared to open field cultivation. The system can support seed germination possess as well which will abolish the need for plant seedling transplanting and
thus reducing production costs. Furthermore, the simplicity and mobility of raft hydroponic system construction make it effortless to construct the system next to the markets thus reducing transport and labour costs, which gives it another advantage over compost-based cultivation. The patented system was suggested for lettuce supported by successful cultivation of Avocado and beans (Farnsworth, 1977). Growing Sweet Basil, in the current analysis, in raft system gave a larger root system, which enhanced plants growth. NFT hydroponic system found to be less effective, for plant species cultivation, in increasing plant material (yield) and in nitrates displacement. Furthermore, the raft hydroponic system showed better results in not only in hydroponic cultivation but in aquaponics cultivation as well (Lennard & Leonard, 2006). In raft hydroponic technique the Sweet Basil roots system was immersed almost completely in the nutrient solution during the full duration of the study but this was not the case for the root system in the ebb and flow technique where the roots were exposed to nutrient solution only in the ebb cycle. Ebb and flow hydroponic system considered costly for wide-scale crop production. However, some researchers demonstrated that Basil does not thrive well in abundance of moisture as the case when grown in a raft floating hydroponic system and rather grows better in NFT hydroponic system. In this regards, NFT can mix the water solution in the hydroponic system rather than a still nutrient solution presented as the raft or floating hydroponic system. While the preference towards NFT is established, although it was found that the cost of wide-scale NFT system and the cleaning requirements for such scale prevents the system to be profitably effective when compared with raft hydroponic system. NFT found to promote high yield for Lettuce (Kowalczyk et al., 2014; Resh, 1995; Sheikh, 2006). This does not agree with the finding in the current study when Sweet Basil gave a high yield in raft hydroponic system compared with NFT. In the raft, hydroponic system adding a simple water/air pump can resolve the issue related to unmixed water/nutrient solution.
The Rockwool hydroponic media considered compatible with plant species rooting system, which is an optimum and dependable ambience for seedling and mature plants growth compared with other mediums including cheesecloth, blue blotter paper, brown germination paper, filter paper, fibreglass matting, agar, and compost- or vermiculite-filled straws. Even when drenched, Rockwool still aerated by about 15%. Rockwool hydroponic media showed better growth indications for Sweet Basil than clay pebbles and coco coir. The Rockwool and coco coir hydroponic medium can maintain water and nutrient solution for long periods of time but the root system is not in the same contact percentage as in the raft hydroponics. Clay pebble hydroponic media has less ability to moisture retain than the rest of the cultivation mediums. The research suggested that Rockwool hydroponic media is the optimum growth media for *Rosa hybrida* L. and it can be recycled for furthermore hydroponic cultivation in the same quality as first time used Rockwool. The Rockwool hydroponic media in a raft hydroponic technique is described by many researchers as simple and easy to sustain hydroponic practices (Gibeaut *et al.*, 1997; Jeong & Hwang, 2000). Clay pebbles or grow rocks is a media with high aerated capability for the root system but requires constant watering. In the current study, clay pebbles is the second choice for optimum Sweet Basil growth. Economically, clay pebbles considered not to be effective media. Many growers consider coco coir soilless culture a sustainable media and promote better growth when mixed with Rockwool in a ratio of 5: 3, Rockwool: coco coir. Furthermore, coco coir considers to economically effective in some regions of the world (coconut is grown in more than 95 countries). It showed better growth for floricultural plants, peppers, strawberry, and eggplants (Cantliffe, Castellanos & Paranjpe, 2007; Faostat, 2019; Resh, 1995; Sheikh, 2006). All this in consideration, coco coir growing media in Raft/Floating system, promoted the lowest value of all the parameters studies in current analysis between hydroponic mediums. This might be to the condense nature of the coco coir media and due to its high water saturation nature which resulted in the minimum
aerated root system that resulted in shorter, smaller and lighter plants (Domeño, Irigoyen & Muro, 2009). Coco coir can be a suitable media if mixed with other soilless cultures such as perlite or Rockwool. Rockwool found to have more aerated capability than coco coir although they have similar water saturation levels. Furthermore, it was reported that fully saturated Rockwool media performs better as a growing culture than half saturated Rockwool (De Rijck & Schrevens, 1998).

Management of fertilization was more manageable in a hydroponic system, unlike compost-grown plants where monitoring nutrient levels instantaneously is a difficult task (Colburn Jr, 1991). Hydroponic cultivation help monitor nutrient levels, PH, and EC easily and top up the nutrients levels, regulate EC and PH by adding the appropriate amount of hydroponic solution. Managing Berries hydroponically and adjusting nutrients, EC and PH had a significant effect on biomass and flavour of plants.

NFT hydroponic technique found to be less effective in promoting high-quality yield for Basil than growing it in deep flow hydroponics which can be explained to the maximum exposure of the root system to the nutrient solution (Walters & Currey, 2015). Raft hydroponic system can assist furthermore adjustment in cultivation proses of plant species such as adding cooling elements in lettuce hydroponic tanks thus helps the grower to hinder seed establishment in the end to development state. Furthermore, the floating hydroponic system enables the vast cultivation areas to be converted into a semi-mechanic production line using conveyers thus minimizing or in some cases eliminating labour costs and increasing production rate (Resh, 1995).

**Conclusion**

Looking at the outcome of both analyses, it concluded that growing methods and alternatives have a significant impact on the yield and both physiological and morphological characteristics
of Sweet Basil (*Ocimum basilicum*) in an enclosed semi-controlled environment. Glasshouse production of Sweet Basil can be enhanced remarkably by switching from compost base cultivation to soilless-water base culture. This shift in growing media preference can be considered sustainable as it shows the minimum use of water in the additional recyclable or reusable capability of the growing media (Diara *et al.*, 2012; Islam *et al.*, 2017; Jeong & Hwang, 2000). Compost based cultivation demanded extra supplementary water compared with the hydroponic growing of Sweet Basil. Rockwool hydroponic media promoted better growth, high light-saturated insentience maximum photosynthetic rate, high yield, taller plants, larger leaves, high fresh/dry weight, and higher value of stomatal conductance and high rate of chlorophyll fluorescence than the rest of cultivation mediums. Comparing hydroponic systems, a raft/floating system promoted high value for all the parameters studied. Combining floating/raft system with Rockwool hydroponic media can be a better method of Sweet Basil production in a controlled environment.
Chapter 3

The impact of light spectrum on growth and development of Sweet Basil (*Ocimum basilicum*) and Bush Basil (*Ocimum minimum*) grown in a controlled environment
**Introduction**

Light is a crucial element for plant growth and production and in areas where solar radiation (natural light) is not sufficient for optimal plant growth, production and supplementary light sources are often provided with high-pressure sodium lamps (HPS) and other light sources used. However, HPS lamps are electrically efficient but generates high radiant heat. As consequence, there has been a lot of interest in replacing HPS lamps with new more efficient lighting sources in the form of light-emitting diodes arrays (LEDs). The effects of three lighting sources (White LED, Blue/Red LED, and HPS) on the growth, development and on the essential oil yield and quality of Sweet Basil and Bush Basil investigated. There was a clear advantage to the Blue/Red LED lighting unit (452 nm (Blue), 632 nm (Red)) on virtually all growth and physiological parameters measured for both Basil species. The HPS lighting system always performed least effective in all comparisons. Combining increases in plant yield and increases in oil yield the Blue/Red LED array outperformed the HPS lights by a factor of approximately double with the white LED being intermediate between these two extremes.

Basil (*Ocimum basilicum* L.) belongs to the *Lamiaceae* family and grows wild in tropical and sub-tropical climates (Makri & Kintzios, 2008). Basil is an important culinary herb and essential oil crop grown and used worldwide (Dube, Upadhyay & Tripathi, 1989; Hossain et al., 2010) and Basil essential oil has been used widely in the food industry as a food flavour, in the medical industries (Simon, Quinn & Murray, 1990), as a component of oral health and dental products and also in the fragrance industry (Kathirvel & Ravi, 2012). Moreover, Basil has been claimed to be effective to treat several medical complaints such as anxiousness, stomach aches, pyrexia, kidney failure, arthropod stings, sickness, infections, headaches, coughs, acne, and constipation (Dasgupta, Rao & Yadava, 2004; Holm, 1999; Kathirvel & Ravi, 2012; Lee et al., 2005; Murugan, Murugan & Noortheen, 2007). In Puerto Rico, North
America, Sweet Basil is used as flatus-relieving medicine for gastrointestinal disorders and found to increase milk production in nursing mothers (Vieira & Simon, 2000).

Basil essential oils contain a wide array of chemical compounds, depending on genotype and growing conditions (light, temperature and irrigation) (Sajjadi, 2006). The main active organic biochemical components of Basil essential oils are; Estragole, a phenylpropene used in perfume manufacturing and as a food additive for flavour (Avetisyan et al., 2017; Smith et al., 2002) and Linalool, which is used widely as a scent in many hygiene products and cleaning agents (Wallace et al., 1991).

Plants do not utilise all wavelengths of white light (sunlight) equally but those between 400 - 700 nm provide photons of the correct energy to drive photosynthesis and these wavelengths are typically referred to as Photosynthetically Active Radiation (PAR) (Britton & Dodd, 1976; Gallo & Daughtry, 1986; McCree, 1972). Other wavelengths are also important in photomorphological development (above 700 nm) and in causing damage to plant cell DNA (below 400 nm) (Sims & Gamon, 2002).

In high latitudes, especially in the northern hemisphere, warm climate crops such as tomatoes, cucumbers and peppers are grown under protected cropping in glasshouses or polygreenhouses and supplementary lighting is necessary in order to maintain sustainable rates of growth and production, especially in the winter when natural daylight is limited (Mitchell et al., 2012). HPS (High-Pressure Sodium) lamps are currently the most commonly used source of supplementary lighting. HPS lamps emit light over a broad, unmodifiable spectrum of wavelengths but with high peaks in the PAR region. Their disadvantage is that they also emit a high degree of infrared radiation leading to thermal emission and raising air temperature and thus reduces the efficiency of these lamps in terms of electrical cost for PAR generation. The
heat production from HPS lamps has been found to have a negative effect on some plant development (Singh et al., 2015).

Recently, LED arrays have surpassed HPS lights in terms of the electrical cost per photon (Jensen, Clausen & Kjaer, 2018; Nelson & Bugbee, 2014) and now LED arrays have a potential for use in agricultural/horticultural production (Bula et al., 1991; Schubert, Cho & Kim, 2000). LEDs have a linear photon output with the electrical input current. LEDs are gaining substantial importance in plant growth industries not only because LEDs can be constructed in arrays large enough to provide sufficient PAR while remaining small in size (few centimetres in diameter) but also emit less heat than HPS lighting lamps (Bosma et al., 2017; Singh et al., 2015; Tonzani, 2009; Van Ieperen & Trouwborst, 2007). LEDs were also known for their durability and long operating lifetime (Dickinson, 2007). Wavelength specificity of LED’s is accomplished via changes in the chemical makeup of the semiconducting material used (Brown, Schuerger & Sager, 1995). An increasing body of research is reporting the role of LEDs in enhancing the shape, edible quality (Lin et al., 2013), biomass, number of leaves (Goins et al., 1997), growth and stem width and other traits of various plant species (Kim et al., 2004b).

Since individual LEDs only emit low levels of light, arrays of several to hundreds constructed to boost the overall light output of lighting devices. Furthermore, each individual LED has a narrow waveband; the construction of arrays provides the manufacturers of lighting devices with an almost infinite combination of different LEDs to produce a unit with a very specific overall wavelength pattern. Many researchers across the world are experimenting with different unit arrays in order to determine which LED combinations are most useful as a supplementary or as a sole source light for plant growth.

The current research aimed to compare three lighting units: HPS, Full Spectrum LED and Blue/Red LED used as a sole source lighting, on the growth and physiological parameters
(plant height, leaf area, fresh and dry weight, stomatal conductance, maximum photosynthesis rate and chlorophyll fluorescence ratio (as a measure of photosynthetic efficiency)) of two species of Basil: Sweet Basil (*Ocimum basilicum*) and Bush Basil (*Ocimum minimum*). Moreover, it aimed to investigate the effect of these lighting units on the quantity and quality of essential oil from Sweet Basil.

**Material and Methods:**

Sweet Basil (*Ocimum basilicum*) and Bush Basil (*Ocimum minimum*) seed obtained from Suttons seeds Ltd (UK). Four replicate pots per species used in each light treatment and with two experimental repeats. The treatments were set up by compartmentalising a controlled environment (CE) room into three compartments and randomly allocating the treatments to the compartments. In the experimental repeat, the treatments re-randomized to the compartments. The pots (9 x 9 cm) used in the experiment were filled with 250 g of compost (John Innes No.1) and four seeds were sown in each pot. Compost was used in this experiment rather than a hydroponic layout due to health and safety issues in the laboratory (the fear of using hydroponic system near non-waterproof electrical sockets as well as lighting units which is a fair hazard).

After sowing the seeds, each pot watered with 1000 mL of water. The pots randomly distributed within each compartment. Temperature and humidity were monitored using Gemini data loggers (Tinytag Plus, No GP-1590- Fisher Scientific). The temperature was set up in the CE room to be 28 °C. The dark/light period was set to 8 H dark/16 H light. The three-light units used; High-Pressure Sodium lamp (HPS) (Omega, UK), White (full spectrum) LED (W LED) (LED Hydroponics, Skyline 400) and Blue/Red (BR) lighting LED (Mars Hydro, Mars II 1600). Light intensity for all of the lighting units (HPS, W LED, and BR LED) was monitored and adjusted using a Skye PAR quantum sensor (Skye Instruments, UK) to provide 470±20 µmol m$^{-2}$ s$^{-1}$ for all treatments at the surface of the culture pots. The light spectra measured and monitored using an UPRtek MK350N premium standalone handheld spectral light meter.
spectrometer (Figure 15). The HPS lamp showed a broad spectrum of wavelengths with the main radiation peaks at 596 nm yellow, W LED showed three main wavelength peaks; 451 nm Blue, 550 nm Green and 620 nm Red with a significant amount of light distributed in the Green region whilst the BR LED had two main peaks 459 nm Blue and Red 632 nm. Growth and physiological parameters measured at two stages of development: at the maximum leaf development stage and at full flowering stage (harvest 1 and harvest 2). The plants continuously monitored and irrigated when needed. Extra air circulation had to be provided to plants grown under HPS light unit to maintain the same temperature as other treatments because of heat gain from the lamp and this meant that more frequent irrigation was needed in these pots. Plants were grown for a total of 48 days. Physiological measurements included; light-saturated instantaneous maximum photosynthetic rate $A_{\text{max}}$ ($\mu$g C/m$^2$/s) measured using an LCi-SD Highly Portable Ambient Photosynthesis System (ADC BioScientific, Herts); chlorophyll fluorescence rate ($Fv/Fm$) measured using a Hansatech Pocket PEA meter (Hansatech Ltd, Norfolk, UK); stomatal conductance ($G_s$ mmol m$^{-2}$ s$^{-1}$) measured using a Delta-T AP4 Leaf Porometer (Delta T Devices, Cambridge UK). Morphological measurements included; plant height (cm) from the compost surface to the top of the plants; leaf area $L_A$ (mm$^2$), using leaf an area image analyser HITACHI KP-D40 colour digital camera with a lightbox and WinDias 1.5 software (Delta-T Devices Ltd); fresh weight $FW$ and dry weight (g) after removing the root system, using a sensitive Fisher Scientific SG-402 laboratory balance; Plants were dried at 60° C for 96 h (Saha, Monroe & Day, 2016).
Chemical analysis

Dry plant material (stems and leaves) was used to extract the essential oil from Sweet Basil using a solvent extraction method (Burbott & Loomis, 1967) with some modifications. Dry plant material (20 g) was grounded in a mortar and pestle with 10 mL of FISHER Scientific’s HPLC grade C₆H₁₄ (Hexane) ≥ 95% (Thermos Fisher Scientific). The resultant mixture was then added to a sintered column with the addition of 60 mL of Hexane. The mixture then drained and the solution collected. A significant amount of used Hexane was evaporated using a BÜCHI R-124 Rotary Evaporator System. A blow-down technique using thermal Techne® Sample Concentrator and BOC Nitrogen gas was applied to evaporate the rest of the Hexane.
from the solution (Zhao, 2004). The essential oils of Sweet Basil were then collected in a vial. The vial was weighed after the blow-down to calculate the quantity of the essential oil obtained.

**Gas Chromatography GC**

Five main components of Basil essential oil, according to their common appearance in the literature (Calín-Sánchez et al., 2012; Joshi, 2014; Leal et al., 2008) and their industrial importance (Market, 2017), were isolated and measured: Estragole C$_{10}$H$_{12}$O, Eugenol C$_{10}$H$_{12}$O$_2$, (Ethyl) E cinnamate C$_{11}$H$_{12}$O$_2$, Eucalyptol C$_{10}$H$_{18}$O and Linalool C$_{10}$H$_{18}$O.

Reference samples of the five compounds were obtained from SIGMA-ALDRICH with a purity of ± 99% and a diluted solution was prepared to a concentration of 250 mg$^{-1}$ mL with Hexane to be compatible with the Gas Chromatograph and were used as standards for the GC analysis. GC analysis was conducted according to the procedure of (Chenni et al., 2016; Kumari & Agrawal, 2011). The GC was performed using a 7890B Gas Chromatograph (GC) System. The essential oil of Sweet Basil (0.025 g) was added to 10 mL Hexane to make a stock solution. A working solution was then prepared by adding 1 mL of the stock solution to 9 mL of Hexane. 1-1.5 mL of the working solution was added to GC vial. Stock solutions of the reference samples were prepared using the same procedure: 0.1 mL of each stock solution of each chemical (5 chemicals in total) added together in a single GC vial to give a total of 0.5 ml. Then 0.5 mL of Hexane was then added to the GC vial to make a final test solution of 1 mL solution of 250 mg$^{-1}$ mL concentration.

1-1.5 of Hexane was added to each GC vial and then analysed using an Agilent Gas Chromatograph 7890A equipped with a 7683 Series Autosampler and 7683B Series Auto-injector [https://www.agilent.com](https://www.agilent.com) and HP5 Column, 5 % phenyl – 95 % dimethylsiloxane, low polarity column, 30 m x 0.320 mm i.d., and 0.25 μm film thickness. The carrier gas was nitrogen at a flow rate = 1.0 mL min$^{-1}$, Injector temperature was 250 °C, Flame Ionisation
Detector temperature was 300 °C, Hydrogen flow rate was 40 mL min\(^{-1}\), Airflow rate = 400 mL min\(^{-1}\), Nitrogen (make-up) flow rate = 15 mL min\(^{-1}\). General Method temperature was programmed at 40 – 300 °C at 10 °C min\(^{-1}\), 10 min at 300 °C using ChemStation software (Revision B.03.01, May 2007).

**Statistical analysis**

Results are presented as means ± standard error (S.E.). All data were subjected to analysis of variance (ANOVA) using Minitab software (version 17) and comparisons of means were made using the least significant difference test (LSD) at 5% level of probability.

**Results**

**I. Physiological responses**

**Maximum Photosynthetic rate** \(A_{\text{max}}\)

There was a significant effect of the lighting treatments on the light-saturated instantaneous maximum photosynthetic rate \(A_{\text{max}}\) of Sweet Basil \((P = 0.005)\) and Bush Basil \((P = 0.001)\). The highest \(A_{\text{max}}\) was observed in plants grown using BR LED lights at both harvest stages. There was no significant impact of the harvest stage and there was no interaction between the lighting treatments and harvest stages on the \(A_{\text{max}}\) (Figure 16).
Figure 19. The effect of light and harvest on $A_{\text{max}}$ of Sweet Basil (LSD=0.87 for light) and Bush Basil (LSD= 0.87 for the light). Different letters denote significant differences (P < 0.001).

**Stomatal conductance Gs**

There was a significant effect of light treatments and harvest stage on the stomatal conductance Gs in Sweet and Bush Basil (P ≤ 0.001). Moreover, there was a significant interaction between the light treatments and harvest stage on Gs for both plant species. The highest Gs was observed under the BR LED lighting unit at both harvest stages and in both plant species (Figure 17).
Figure 20. The effect of light and harvest on $G_s$ in Sweet Basil (LSD = 0.144 for the light, LSD = 0.118 for the harvest and LSD = 0.204 for the interaction between light and harvest) and Bush Basil (LSD = 0.13 for the light, LSD = 0.11 for the harvest). Different letters denote significant differences between treatments. ($P < 0.05$).

**Chlorophyll Fluorescence Rate Fv/Fm**

There was a significant effect of the light treatments on the chlorophyll fluorescence rate Fv/Fm for Sweet Basil ($P = 0.001$) but not for Bush Basil ($P = 0.1$). The highest Fv/Fm was observed in plants grown under the BR LED light treatment in Sweet Basil for both harvest stages (Figure 18). There was a significant effect of the harvest stage on Fv/Fm in Bush Basil ($P = 0.02$) and Fv/Fm at the first harvest was significantly higher than at the second harvest. However, for Sweet Basil, the harvest stage had no significant effect on Fv/Fm ($P = 0.2$).
Figure 21. The effect of light and harvest on Fv/Fm in Sweet Basil (LSD = 0.042 for the light) and Bush Basil (LSD = 0.05 for the harvest). Different letters denote significant differences between treatments ($P < 0.05$).

II. Morphological responses

Height

There was no significant effect of the light treatments on plant height in both Sweet and Bush Basil. The harvest stage has a significant effect on the plant height ($P \leq 0.001$) with taller plants observed at harvest stage two (Figure 19).

Figure 22. The effect of light and harvest on Sweet Basil height (LSD = 2.06 for harvest) and Bush Basil (LSD = 2.4 for harvest). Different letters denote significant differences between treatments ($P < 0.001$).
Leaf area LA

In Sweet Basil, there was a significant effect of the light treatments and harvest stage on the leaf area LA ($P \leq 0.001$). The optimum LA was observed in plants grown under the BR LEDs at both harvest stages for both species. For Bush Basil, there was a significant effect of the light treatments ($P = 0.02$) on LA and plants cultivated under BR LEDs showed larger leaf area per ground surface area unit. LA was significantly higher ($P \leq 0.001$) at harvest stage two in both plant species (Figure 20).

![Figure 23](image_url)

**Figure 23.** The effect of light and harvest on LA of Sweet Basil ($\text{LSD} = 663.1$ for the light, $\text{LSD} = 541.4$ for the harvest) and Bush Basil ($\text{LSD} = 68.4$ for the light, $\text{LSD} = 55.9$ for the harvest and $\text{LSD} = 96.8$ for the interaction between light and harvest). Different letters denote significant differences between treatments ($P < 0.001$).

**Fresh weight FW**

There was a significant impact of light treatments on the fresh weight FW of Sweet Basil ($P = 0.006$). The maximum FW was observed in plants grown under BR LED lighting unit at both harvest stages for both plant species. There was a significant effect of the harvest stage ($P \leq 0.001$) on FW. At harvest stage two FW was significantly higher in comparison with harvest stage one for both Sweet and Bush Basil. In terms of Bush Basil, there was a significant interaction between light treatments and harvest stage ($P = 0.01$). While the highest fresh
weight was observed in plants grown under White LEDs at harvest stage one, FW was highest in plants grown under BR LEDs at harvest stage two (Figure 21).

Figure 24. The effect of light and harvest on FW of Sweet Basil (LSD = 5.2 for the light treatment, LSD = 4.3 for the harvest stage) and Bush Basil (LSD = 4.4 for the harvest stage and LSD = 7.7 for the interaction between the light treatments and harvest stage). Different letters denote significant differences between treatments (P < 0.001).

**Dry weight DW**

There was no significant impact of the light spectrum on DW of Sweet Basil but there was a significant effect on Bush Basil (P = 0.001) under BR LEDs (Figure 22). The harvest had a significant impact on DW for both species and was significantly higher at harvest stage two.
Figure 25. The effect of light and harvest on DW of Sweet Basil (LSD = 1.5 for the harvest stage) and Bush Basil (LSD = 1.6 for the lighting treatment, LSD = 1.3 for the harvest stage and LSD = 2.3 for the interaction between the lighting treatments and harvest stage). Different letters denote significant differences between treatments ($P < 0.001$).

**Essential oil yield and composition**

Light treatments had a significant impact on the yield of essential oil in Sweet Basil ($P = 0.005$). The highest essential oil yield was observed in plants cultivated under BR LEDs (Figure 23).

![Essential oil yield](image)

Figure 26. The effect of light treatments on the yield of essential oil in Sweet Basil (LSD = 0.049). Different letters denote significant differences between treatments ($P < 0.001$).

Results from the Gas Chromatography analysis showed that LED lighting significantly improved the accumulation of Linalool, Eugenol and E-Cinnamate in the essential oil of Sweet Basil compared to the accumulation of those biochemicals in the essential oil of plants grown under HPS lamp. Moreover, BR LEDs significantly increased the concentration of Eucalyptol in contrast with both White LED and HPS lights (Figure 24).
Figure 27. The effect of light on the composition of essential oil in Sweet Basil (LSD = 17.3 for the lighting treatment). Relative abundance shows the average intensity of biochemical signals. Different letters denote significant differences between treatments ($P < 0.001$).

Discussion

This study clearly demonstrated that different lighting systems with the same PAR output but with different spectral profiles can influence the growth and yield of Basil plants and the yield of essential oil. This correlates well with several other studies (Currey & Lopez, 2013; Gómez et al., 2013; Pimputkar et al., 2009; Randall & Lopez, 2014). A consistent and strong trend can be observed through all of the results in favour of LED arrays in comparison to HPS lights and in favour of the BR LED in comparison to the White LED array. The physiological
measurements made indicated that photosynthesis was more productive under the LED arrays with higher $A_{\text{max}}$ and Fv/Fm and this appears to have resulted in greater Leaf Area production leading to greater growth and yield. Gaining this sort of knowledge of plants growth and morphology in response to lighting changes gives a better understanding of more efficient cultivation of these plants. In particular, controlled environment cultivation of plants increases the chances of enhancement and uniformity in yield and quality but standard controlled conditions for each plant species needs to be carefully optimised. (Chen & Chory, 2011). Precise regulation of environmental control in commercial controlled environments can be considered to still be in its early stages since each plant species may have a different response to different light quality and spectra, photoperiods and light integrals as it passes through its developmental stages.

These considerations must be observed when switching the cultivation of plants from field to any indoor farming unit and monitoring the photosynthetic activity is an important tool to achieve that goal. Mimicking the sunlight energy efficiency, when sunlight is not available, is not an easy task but has been established that the effect of Red light in the region of 650-665 nm from LED lighting units matches the assimilation peak of the photoreceptors Phytochrome and Chlorophyll. It was shown also, that the combination of Red-Blue light in LED lighting for growing plants can enhance the Maximum Photosynthetic rate $A_{\text{max}}$ as a consequence of activation of Cryptochromes, Phytochromes and Chlorophyll more than if a monochromic light was used as a grow light (Darko et al., 2014). This increase in $A_{\text{max}}$ was explained as a result of the increase in carbon dioxide levels when the Blue light caused a better stomatal gapping (Assmann & Shimazaki, 1999; Doi, Kitagawa & Shimazaki, 2015; Inoue & Kinoshita, 2017). The addition of Blue LED light in the region (380-495 nm) also stimulates Chlorophyll and is also found to trigger Cryptochromes influences stem growth. This correlates with the results presented here where it was found that $G_s$ was higher under BR LED. This increase in $A_{\text{max}}$
and Gs thus leads to an increase in DW with the addition of Blue light. The value of $A_{\text{max}}$ can respond to the Red light (peak 656 nm) more than a response to a White LED light (Tennessen, Singsaas & Sharkey, 1994). On the other hand, it was found that there is a negative effect of the addition of Green LED light (495-570 nm) on the growth and development of plants which supports our findings as White LED and HPS lighting (contain some Green spectrum) promoted lower values of growth than those plants under BR LED light (no green light). Curiously, PAR is defined to include all wavelengths between 400 and 700 nm which of course includes green light which is yet to be proven to be photosynthetically active. This challenges the set-up of experiments designed to compare lighting units with each other. If for example, as used here, the PAR is set to be similar under all light units, by the above definition, those containing green light will be proportionately lower in the required red and blue light. Such considerations may require a re-examination of the instruments used to measure light in LED-lit controlled environments with a possible move away from Quantum Sensors towards Spectrometers which can measure specific wavelength intensities more accurately.

In the process of photosynthesis, Photosystem II (PS II) light-dependent chemical reaction productivity rate (Fv/Fm) can be measured as an indication of a healthy plant and typically a healthy plant leaf will measure around 0.83 (Baker & Oxborough, 2004; Strasser, Srivastava & Tsimilli-Michael, 2000). Fv/Fm is sensitive to the light conditions and therefore lower values of Fv/Fm can indicate stress via photo-inhibition. Fv/Fm is related to the quantum yield in PS II as other studies have shown similar results where Blue LEDs increased photochemical quenching whilst Red LEDs enhanced chlorophyll content despite chloroplasts being smaller (Wang et al., 2015). The current analysis has shown that a combination of Blue (459 nm) and Red (632 nm) LED lights can promote a higher Fv/Fm than HPS or white LED lights of the same PAR output. Moreover, Fv/Fm was around 0.8 (optimal) even at harvest stage two using the BR LEDs (Figure 2) whereas it had diminished to less than optimal under the other light
treatments. This is an indication that the BR LEDs provides suitable conditions for efficient photosynthetic activities to persist at the late growth stage of the plant (Darko et al., 2014; Muneer et al., 2014; Naznin et al., 2019).

The effects of light quality on photosynthesis go parallel with the effect on stomatal gapping. It has been established that Blue region of the spectrum has more impact on the stomatal opening than Red light (Lurie, 1978; Sharkey & Raschke, 1981). Both of these studies showed the low impact of green light on stomatal opening. Red and blue wavelengths are both utilised during photosynthesis by chlorophyll and secondary pigments, therefore promoting growth and development in plants (Muneer et al., 2014; Wang et al., 2015). A recent study sought to unpick the influences of Red and Blue light on growth and photosynthetic efficiency in Sweet Basil and found that quantum efficiency in PSII was lower under Red/Blue LED light arrays when the Red portion of the light array was greater than double the Blue (2:1). This was reversed when light ratios were more equal (1:1) (Pennisi et al., 2019). It is the presence of the Photosystem II Primary Donor protein (Pigment 680) P680 in PSII which absorbs maximum light energy at 680 nm (Red) and Photosystem I Primary Donor (Pigment 700) P700 in PSI which absorbs maximum light energy at 700 nm (Red) (Hall et al., 2013) and these might be responsible for the effects on the stomatal conductance Gs (Durrant et al., 1995; Scarth, 1932; Webber & Lubitz, 2001). In the current study, the ratio of Blue; Red in BR light treatments was higher than the ratio of Blue; Red in both White LED and HPS lighting units. This could explain the high Gs value results under BR LED as a response to the high Blue; Red ratio.

An increase of Blue light had a positive effect on lettuce a negative impact on soybean and wheat (Dougher & Bugbee, 1998). In contrast, Sweet Basil was positively affected by Blue light when measuring FW (at 200 µmol m⁻² s⁻¹) and when the same light quality compared with low light intensity (at 100 µmol m⁻² s⁻¹) the FW value was lower indicating the importance of combinations of wavelengths and quantity to promote high yielding plants (Dou et al., 2017).
Light has a relationship with FW of plant material and it has been found that Blue light (455-475 nm) with light intensity (140±10 µmol m$^{-2}$ s$^{-1}$) resulted in high FW (Gök, Bekir & Bayhan, 2016).

The highest LA value was observed under Blue/Red lighting treatments in comparison with both white LED and HPS lighting units. In agreement with current findings, the light spectrum of 580 nm, 600 nm was reported to inhibit the growth and development of leaves in Lettuce leading to a low value of LA (Kim et al., 2004a). However, in disagreement with the current research, it was reported that an increase of Blue light 455 nm by 6.7 µmol m$^{-2}$ s$^{-1}$ over 400 µmol m$^{-2}$ s$^{-1}$ for 20 hours decreased the LA of tomato and Cucumber (Ménard et al., 2005). The difference in plant heights and LA response to light quality could be due to the effect of plant species and experimental conditions.

Alongside the main photo-related photosynthesis reaction, a phenyl based anabolism reaction accrues in plant species resulting in organic matter that gives plants their specific aroma and taste. Many research reports have indicated the impact of light quality on the essential oils of plant species (Amaki et al., 2011a; Chang, 2005; Fahlén, Welander & Wennersten, 1997; Skrubis & Markakis, 1976) and the current data agrees that light composition has an effect on the quantity and quality of essential oil of Sweet Basil. In another study, the essential oil content of Basil grown under Blue light has been found to be up to 4 times higher than those grown without Blue light (Amaki et al., 2011a). This is in agreement with our findings where BR LEDs had a positive impact on the level and concentration of important biochemicals in the essential oil of Sweet Basil.

**Conclusion**

Light quality had an impact on the physiological and growth characteristics of Sweet Basil (*Ocimum basilicum*) and Bush Basil (*Ocimum minimum*). LEDs lighting devices had an
advantage in promoting high yielding plants with a higher concentration of essential oil compared to traditional HPS lamps. There was a clear advantage of using a Blue-Red LED lighting unit (452 nm Blue 632 nm Red) compared to a white LED unit; this research could have a great practical application for the commercial production of Basil in sole source controlled environments. Further research is recommended to determine more precisely the optimal wavelength of light in both the Blue and Red regions and the optimal Blue/Red combination for the growth of and production of Basil.
Chapter 4

The effect of specific light wavelengths on the growth and characteristics of Sweet Basil (*Ocimum basilicum*) at different vegetative stages cultivated in a plant factory.
Introduction

Sweet Basil (*Ocimum basilicum*) cultivation using grow lights in indoor farming layouts has been and still is the focus of many researchers (Carvalho et al., 2016; Goto, 2012). In the aspect of choosing the right quality of light for the cultivation of any crop and in this case for Sweet Basil, many researchers refer to the McCree Curve (Eyehortilux, 2019; McCree, 1972) in their work regarding the use of artificial lighting for the cultivation of plant species (Gross, 1982; Ögren & Evans, 1993; Terashima et al., 2009). The McCree curve sheds light on the importance of some light regions in the reaction of photosynthesis. Furthermore, the McCree study was conducted under low light intensity (approx. 50 µmol m$^{-2}$ s$^{-1}$) which has a big limiting impact on the growth and development of plants as demonstrated by (Fan et al., 2013; Lichtenthaler et al., 1981) and others. The McCree curve does not give a full light requirement profile of plants and there still exists the need for a full analysis to determine individual plant species light requirements. In the current study, Sweet Basil (*Ocimum basilicum*) was chosen as a model for this analysis. Sweet Basil was cultivated under controlled environment conditions (temperature, humidity and light) in a plant factory system and grown hydroponically. Several light treatments were applied including combinations of blue and red LEDs under 14-hour photoperiod.

Aims and objectives

The experiment aimed to investigate whether supplying a light regime that matches plant absorbance would improve the growth and quality of Sweet Basil. The influence of specific wavelengths of light on the growth of Sweet Basil including leaf area, photosynthetic rate and stomatal conductance were investigated in a controlled environment.
Material and Methods:

The absorption spectrum of Sweet Basil

Plant material for pigment extraction was obtained from Skardon gardens at the University of Plymouth. Sweet Basil cv. Maggie seeds were obtained from CN seeds (https://cn-seeds.co.uk) and sown and grown in a glasshouse where the light condition was full daylight (February 2019) plus supplementary High-Pressure Sodium (HPS) lamps.

In the glasshouse the average temperature was 18 ± 2 °C, the humidity was 56 ± 2%, the light condition was full daylight (June-September 2018) plus supplementary lighting, using High-Pressure Sodium (HPS) lamps, that programmed to turn on when the ambient lighting becomes lower than 10 µmol m\(^{-2}\) s\(^{-1}\) (7 p.m. - 7 a.m.). HPS lamps in the greenhouse provided 12 ± 2 µmol m\(^{-2}\) s\(^{-1}\) supplementary light intensity and the thermal screen was programmed to cover the glasshouse at 24 °C during daytime and 10 °C during night-time. Vents were programmed to open at 22°C during the day and 19°C during the night. Heating was set at 18 ± 22 °C with humidity threshold of 65%. The pots used for the cultivation were 9 x 9 cm and 250 g of compost (John Innes No.1) per pot was used. After sowing the seeds, each pot was watered with 1000 mL of water and then whenever needed. The pots were randomly distributed. Temperature and humidity were monitored using Gemini data loggers (Tinytag Plus, No GP-1590- Fisher Scientific). The plants were harvested at full vegetative growth (harvest stage, pre-flowering) and were 20 cm tall.

Two experimental replicates from three separate Sweet Basil plants were used (three biological replicates). Pigments were extracted by grinding 0.2 g of plant leaves in 10 mL 80% acetone using a mortar and pestle. The resultant solution was made up to 25 mL using an additional 80% acetone then centrifuged for two minutes using a ROTOFIX 32-An https://www.hettichlab.com/en/product/rotofix-32-a/ at maximum revolutions per minute of 6,000 RPM min\(^{-1}\). After centrifugation 2 mL of the solution was scanned in a Jenway 7315
spectrophotometer, measuring the absorption of light between 300 nm and 700 nm with the absorption taken at 5 nm intervals. The resultant data were used to create the light absorption curve for Sweet Basil (Figure 27).

Figure 28. Spectrometric analysis of Sweet Basil (Ocimum basilicum) pigment absorption using Jenway 7315 spectrophotometer. Results presented as means ± standard error (S.E.).

The absorption curve showed three main absorption peaks, an ultraviolet peak at 330 nm (shoulders from 300 to 360 nm), a broad blue peak at 435 nm (shoulders from 400 to 500 nm) and a red peak at 663 nm (shoulders from 640 to 690 nm).

The assessment of growth of Sweet Basil under three-light LED regimes

Sweet Basil cv. Maggie seeds were obtained from CN seeds (https://cn-seeds.co.uk) and sown and germinated in the greenhouse (the average; temperature was 18 ± 2 °C, humidity was 56 ± 2%, the light condition was full daylight plus supplementary lighting, using High-Pressure Sodium (HPS) lamps, that programmed to turn on when the ambient lighting becomes lower
than 10 μmol m⁻² s⁻¹ (7 p.m. - 7 a.m.). HPS lamps in the greenhouse provided 12 ± 2 μmol m⁻² s⁻¹ supplementary light intensity and the screen was programmed to cover the glasshouse at 24 °C during daytime/ 10 °C during night-time. Vents were programmed to open at 22°C during the day and 19°C during the night. Heating was set at 18-22 °C with humidity threshold of 65%. At Skardon gardens (University of Plymouth) April 2019 in Plymouth - UK, Max solar energy average was approx. 650 μmol m⁻² s⁻¹, Sunshine hours was 129.5 h, the average temperature was 25.7°C, Solar kilowatts per hour was 115.4 kWh. 

https://www.bearsbythesea.co.uk. Seedlings with one pair of leaves then transferred to the plant factory in the University of Plymouth. The Plant Factory is a converted greenhouse where external light has been excluded and a multi-tier hydroponic growing space has been installed with interchangeable LED light units. The temperature and humidity were monitored using Gemini data loggers (Tinytags Plus (part No GP-1590)) and an instantaneous thermometer (Fisher Scientific) at 28 ± 2 °C. The dark/light period was set to 8/16 H. Four light treatments were designed and applied:

- An LED light treatment with a combination of red (663 nm) LED light and blue (450 nm) LED light, with a ratio of 1:1.6; this treatment was designated (R/B450). Blue LED light with a wavelength peak at 450nm is commonly used in most current commercially available grow lights (for example see the Osram blue LED lights https://www.osram.com).

- An LED light treatment with a combination of red (663 nm) LED light and blue (435 nm) LED light, with a ratio of 1:1.6; this was chosen to mirror the ratio of absorption from the Sweet Basil absorption curve (Figure 1). This treatment was designated (R/B435). The LED unit in this treatment was manufactured specifically for this analysis.
- An LED light treatment with a combination of red (663 nm) LED light and blue (450 nm) LED light, with a ratio of 1:1. This treatment was designated (R: B; 1:1).

- A Glasshouse light treatment with supplementary HPS light. This treatment to be labelled (GH).

Light intensity emitted from the lighting units was measured using a Skye PAR (Photosynthetically Active Radiation) quantum sensor (calibrated August 2017) and each light panel in the Plant Factory was adjusted to emit a Photon Flux Density (PPFD) of 300 ± 20 µmol m\(^{-2}\) s\(^{-1}\) (Bantis, Ouzounis & Radoglou, 2016) in order to attempt to standardise the PAR growing regime under each light source. The emitted light spectrum of the light units was measured using an UPRtek MK350N premium Standalone handheld spectral light meter and the spectra obtained (Figure 28).

<table>
<thead>
<tr>
<th>R/B450</th>
<th>R/B435</th>
<th>R: B; 1:1</th>
<th>GH</th>
</tr>
</thead>
</table>

Figure 29. Spectra of the lighting in cultivate Sweet Basil obtained using an UPRtek handheld spectrometer (GH spectrum measured on a sunny day).

Physiological and morphological responses of Sweet Basil to the light treatments were measured at three stages of development; at the initial vegetative stage when Sweet Basil plants had 4-5 sets of leaves (Stage 1), end of the vegetative stage when flowering buds started to appear (Stage 2) and at full flowering stage (Stage 3). The experiment was carried for a total
of 40 days (final harvest day 30th of April 2019). Light saturated instantaneous Photosynthetic rate $A_{\text{max}}$ was measured using an LCi-SD Highly Portable Ambient Photosynthesis System (ADC BioScientific, Herts). The $A_{\text{max}}$ was measured from the upper part of the plants (fully developed leaves) in the middle of the day. $A_{\text{max}}$ was measured in microgram per Carbon per square meter per second (µg C/m²/s). Chlorophyll fluorescence rate $Fv/Fm$ is used to indicate the “health” and efficiency of the photosystem in plant leaves and a figure of over 0.8 is considered to indicate zero stress. In this experiment, $Fv/Fm$ was measured using a Hansatech Pocket PEA meter (Hansatech Ltd, Norfolk, UK) in the upper fully developed leaves but different plant leaves chosen than those used to measure $A_{\text{max}}$. Stomatal conductance $G_s$ measurement is indicative of instantaneous transpiration rates indicating how quickly water is being transpired from the leaf and was measured using a Delta-T AP4 Leaf Porometer (Delta T Devices, Cambridge UK). The $G_s$ was measured, in centimetre per reciprocal second (cm s⁻¹), from fully developed leaves but different leaves than those chosen for $A_{\text{max}}$ or $Fv/Fm$. Plant height was measured from the end of the root system to the top of the plants and measured in centimetres (cm) using a ruler. Leaf area $L_{\text{A}}$ was measured, in square millimetres (mm²), using a leaf area image analyser $L_{\text{A}}$; HITACHI KP-D40 colour digital camera with a lightbox and WinDias 1.5 software from Delta-T Devices Ltd. Stem diameter $S_{\text{D}}$ was measured using Vernier Calliper https://www.fishersci.ca/shop/products/plastic-vernier-caliper/s12912 from the point upper the end of the root system. Fresh weight $F_{\text{W}}$ was measured, after removing the root system, using a sensitive Fisher Scientific SG-402 laboratory balance. Dry weight $D_{\text{W}}$ was measured, using a sensitive Fisher Scientific SG-402 laboratory balance, after plants were dried at 60°C for 96 h (Saha, Monroe & Day, 2016). Both $F_{\text{W}}$ and $D_{\text{W}}$ was measured in grams (g).
Figure 30. The layout of the plant factory.
Statistical analysis
Results are presented as means ± standard error (S.E.). All data were subjected to analysis of variance (ANOVA) using Minitab software (version 17) and comparisons of means were made using the least significant difference test (LSD) at 5% level of probability.

Results

I. Physiological responses

Light saturated maximum photosynthetic rate $A_{\text{max}}$

There was a significant effect ($P \leq 0.001$) of the light treatments on the light-saturated instantaneous maximum photosynthetic rate $A_{\text{max}}$. Light treatment R/B435 (Figure 29) promoted the highest $A_{\text{max}}$ and this was the lowest in the glasshouse grown plants. Development stage also had a big impact ($P \leq 0.001$) on $A_{\text{max}}$ and peaked when the plants were at the end of the vegetative stage. Furthermore, there was a significant interaction between the light treatments and the development stages on $A_{\text{max}}$ ($P = 0.007$). Among the plant factory treatments, the light treatment R/B435 had the highest $A_{\text{max}}$ at each of the development stages.

Figure 31. The effect of light and development stage on $A_{\text{max}}$ of Sweet Basil (LSD = 0.3 for the light treatments, LSD = 0.34 for the vegetative stage and LSD = 0.6 for the interaction between the light treatments and the development stage).
Chlorophyll Fluorescence Rate Fv/Fm

Light treatments had a significant impact on Fv/Fm. The plant factory light treatments had significantly ($P \leq 0.001$) higher value of Fv/Fm than the greenhouse at all development stages (Figure 30) and the light treatment R/B435 had the highest Fv/Fm values. Plant development stage also had a significant effect ($P \leq 0.001$) on Fv/Fm increasing as development progressed under all light regimes. There was an interaction between the development stages and the light treatments ($P \leq 0.001$) with more variation under the glasshouse conditions and the B/R; 1:1 treatment than under the R/B450 and R/B435.

![Graph showing Fv/Fm values for different light treatments and development stages.](image)

**Figure 32.** The effect of light and development stage on Fv/Fm of Sweet Basil (LSD = 0.01 for the light treatments, LSD = 0.011 for the development stage and LSD = 0.02 for the interaction between the light treatment and development stage).

Stomatal conductance Gs

There was a big effect of the various light treatments ($P \leq 0.001$) on Gs with the R/B435 treatment showing the highest Gs values and the glasshouse the lowest (Figure 31). The development stage also had a significant effect ($P \leq 0.001$) on Gs progressively increasing with the later development stage. There was no significant effect of the interaction between the light treatment and development stage ($P = 0.18$). Overall, the plant factory light treatments had a
more positive impact on Gs than the glasshouse and the 1:1.6 (R/B) ratio were better than the 1:1 (R/B).

![Figure 33. The effect of light and development stage on Gs of Sweet Basil (LSD = 0.064 for the light treatment, LSD = 0.074 for the development stage.](image)

**II. Morphological responses**

There was a significant effect of the lighting treatments ($P \leq 0.001$) on the height (Figure 32), Stem Diameter (Figure 33) and Number of leaves (Figure 34) with the R/B435 light treatment giving the biggest and more robust plants. Plant size also increased with the vegetative stage ($P \leq 0.001$) but also increased with the lighting treatment ($P \leq 0.001$). Plants grown under the plant factory light treatments were overall bigger and leafier than those grown in the glasshouse and within the plant factory, the light treatment R/B435 gave the biggest plants at each and every development stage.
Figure 34. The effect of light and development stage on H of Sweet Basil (LSD = 0.98 for the light treatments, LSD = 1.13 for the development stage and LSD = 1.96 for the interaction between the light treatments and development stage).

Figure 35. The effect of light and development stage on SD of Sweet Basil (LSD = 0.04 for the light treatments, LSD = 0.05 for the development stage and LSD = 0.09 for the interaction between the light treatments and development stage).
Figure 36. The effect of light and development stage on NoL of Sweet Basil (LSD = 2.5 for the light treatments, LSD = 2.8 for the development stage and LSD = 5.01 for the interaction between the light treatments and development stage).

Leaf area LA

There was a significant effect of the light treatments on the Leaf area LA (P ≤ 0.001) (Figure 35) and the light treatment R/B 435 showed an almost seven-fold increase in LA compared with the plants grown in the glasshouse. Within the plant factory, the light treatment R/B435 had the greatest LA compared to the other light treatments.

Figure 37. The effect of light and development stage on LA of Sweet Basil (LSD = 66.6 for the light treatments, LSD = 76.9 for the development stage and LSD = 133.3 for the interaction between the light treatments and development stage).
Fresh weight (FW) and Dry weight (DW)

The pattern of results of the biomass yield, both Fresh weight FW (Figure 36) and Dry weight DW (Figure 37) were similar to all other parameters measured with the plant factory grown plants exceeding those grown in the glasshouse. The R/B432 light treatment was the highest within the plant factory for both FW and DW ($P \leq 0.001$).

Figure 38. The effect of light and development stage on FW of Sweet Basil (LSD = 5.4 for the light treatments, LSD = 6.2 for the development stage and LSD = 10.8 for the interaction between the light treatments and development stage).

Figure 39. The effect of light and development stage on DW of Sweet Basil (LSD = 0.4 for the light treatments, LSD = 0.5 for the harvest stage and LSD = 0.9 for the interaction between the light treatments and development stage).
Discussion

The data in this study indicate the possibility of yield enhancement for plant species in a controlled enclosure environment (plant factory) in contrast with glasshouse cultivation. To begin with, growing plant species in farmlands has many issues and problems such as less than optimum growing conditions and the outbreak of pests and diseases (Gamliel & Yarden, 1998; Hoang et al., 2005). The cultivation of Sweet Basil under tunnels in Europe was found to be more preferable to produce a high yield (Bączek et al., 2019). Also, it was found that the cultivation of 24 cultivars of Basil in farmland versus a shaded enclosure resulted in high average levels of protein in plants grown under the shaded enclosure (Murillo-Amador et al., 2013). Basil plant quality and biomass were also enhanced when cultivated under an adjustable roof greenhouse compared to field cultivation (Nelkin & Schuch, 2004). In a study on the effect of environmental and development conditions on Basil quality, it was found that greenhouse cultivation would enhance the quality of Basil (Tsasi et al., 2017). On the other hand, the level of Carotenoids and Chlorophyll pigments of eight cultivars of Sweet Basil were higher in open field conditions but there was an accumulation of Carotenoids in Sweet Basil both in the greenhouse and in the open field condition (Kopsell, Kopsell & Curran-Celentano, 2005). Others have also found that the quality of Sweet Basil could be enhanced by cultivation in open fields (Morales, Simon & Charles, 1993). Shading can have a direct effect on the quality of Sweet Basil. Long exposure of Basil plants to solar irradiation increased levels of accumulation of Linalool and Eugenol in Sweet Basil (Chang, Alderson & Wright, 2008). All this confirms the advantages of Sweet Basil cultivation in open field verses an enclosure environment (plant factory/ glasshouse) but yield limitations of open field systems frequently force growers into controlled or semi-controlled growing environments.

The physiological, anatomical and yield measurements made in the current experiment all point to a similar pattern, that the Plant Factory light regimes were significantly better in terms of
growth than the glasshouse grown plants. This could be explained by an inability to adequately optimise the growing conditions in the glasshouse related to the time of year the experiment was conducted but this reflects a commercial reality where the majority of Sweet Basil plants are currently being raised. Growing Sweet Basil in glasshouses with sole dependent on daylight would limit production as the seasonal light conditions altered. In the United Kingdom, the solar irradiation at April is not at a peak as it is at June and it depends as well on where the glasshouse is located which has a significant impact on the solar irradiation in the same month (Burnett, Barbour & Harrison, 2014; Pearson et al., 2006). Furthermore, there are many other factors affecting the cultivation of plant species in a greenhouse such as irrigating system, fertilizing, pest control and labour which can be more manageable in a plant factory cultivation system (Singh et al., 2017). The Plant Factory with its semi-controlled environment of a more constant temperature and consistent lighting was able to provide higher quality plants with a higher yield than the glasshouse in wintertime in the northern hemisphere. Modern control theory can be applied to enhance the performance of the plant factory. Growing tobacco in a plant factory showed the importance of controlling the water status and its interaction with light intensity and quality. Furthermore, frequent discarding and supplementing of water and nutrients can have a direct effect on $A_{\text{max}}$ in the plant factory which needs to be taken into consideration (Morimoto, Torii & Hashimoto, 1995). Such findings endorse the suggestion that Plant Factories can alleviate slow production times associated with conventional propagation methods (glasshouses). In the aspect of the proficiency of asset use, a plant factory, with a multi-tier hydroponic system in a completely enclosed structure with air and temperature controlling unit, can be found to be more cost-effective than using a glasshouse for plant cultivation. Adding LEDs in the plant factory laterally as a boost for the lower leaves of plant species has been found to increase $A_{\text{max}}$ and LA by threefold thus increasing yield and possibly of enhancing quality (Kozai, 2013a). Clearly, the Plant Factory grown plants in the current
experiment were producing more leaves on taller and thicker stemmed plants which had higher photosynthetic rate and higher photosynthetic efficiency. One of the consequences of the greater photosynthesis was a higher transpiration rate (stomatal conductance) but there was no adverse consequence to this as the roots had constant access to unlimited water through the hydroponic system. The high transpiration rate (stomatal opening) in the plant factory is due to the stimulation of both Cryptochromes and Phototropins in Sweet Basil by providing a combination of blue and red light. This combination of light spectra in the plant factory can enhance photo-tropism, circadian clock and inhibit etiolation in plants (Massa et al., 2008). In contrast, controlled environment cultivation of lettuce did not have any positive impact on the stomatal conductance. This could be explained either due to the fact that the lettuce is different plant species than Sweet Basil and as a result has different responses to the environmentally controlled conditions or due to the fact that lettuce plants were grown under red, blue and white LEDs with low light intensity and broad wavelengths for red and blue LEDs. Kang et al., 2013 suggest that a high level of CO₂ inside the plant factory might neutralize low or inefficient lighting (Kang et al., 2013). In the relation to the current study, the CO₂ levels are one of the elements that can be investigated in the future as a factor to optimise and enhance the yield and quality of Sweet Basil and other pharmaceutical plant species as there may well be an interaction with LEDs. Furthermore, it has also been found that delaying the illumination of red light in a blue: red combination increased FW significantly in lettuce (Jishi et al., 2016). This can be an additional step to enhance the performance of the plant factory. It was found that DW and LA inversely proportional to the increase of blue light (450 nm) in pepper, tomato and cucumber but not for wheat, lettuce and soybean. However, it was confirmed that this increase of blue light has to be limited to a certain point otherwise the DW and LA will decrease (Snowden, 2015). This highlights the focus of research on the percentage of the blue light in the cultivation conditions rather than the impact of different wavelengths of the blue light on
plant species. A study on the action and quantum yield spectra of photosynthesis for 33 plant species established that all species curves showed a significant high and wide peak at red light region 600 to 680 nm and low peak and shoulders at the blue light region at 435 nm. Furthermore, there was a significant co-relation of green light at 560 nm on the ratio action of the blue light at peak 435 nm in all plant species tested. This is the only study to clearly show the relative importance of blue light at 435 nm over 450 nm (Inada, 1976). The importance of blue light at 435 nm has also been shown to stimulate PS I in the photosynthesis process in Cyanobacteria Bactria and Arabidopsis thaliana (Lamb, Røkke & Hohmann-Marriott, 2018). In Bean plants, the action spectrum analysis curve showed a blue light peak at 437 nm (Balegh & Biddulph, 1970). A fluorescence spectra analysis showed that Chamomile pollen has a peak at a blue light region of 435 nm (Roshchina, 2008).

It has been established that blue light provided for plant growth and development is absorbed and utilized by Cytochromes and Phototropins, more especially blue light at the region of 420-450 nm is utilized by Cytochrome enzyme P450. In general, Cytochromes are enzymes that control the electron transfer chain but need to be activated by blue light. P450 was found involved in many oxidation reactions in the cellular level of plants, spatially it was found to be involved in the synthesizing process of biochemicals such as Phytoalexins, terpenes, and phenols. It was found also there are multiple types of P450 enzyme in plants (P420, P680, and P700) that respond to different wavelengths of light spectra. Furthermore, although it was established that there are 244 cytochrome P450 genes, studies suggested that there is more to be discovered in the genetic level related to P450 enzymes. This great genetic abundance of the P450 family highlights the importance of blue light in plant growth and development (Donaldson & Luster, 1991; Morant et al., 2003; Werck-Reichhart & Feyereisen, 2000). The enzyme P435 as an enzyme that is stimulated by blue light at a peak wavelength of 435 nm has only been identified in photoautotrophic Gram-negative bacteria but yet to be identified in
plants (Seibert & DeVault, 1971). Yet in the current study 435 nm was found to be superior to 450 nm illustrating that a clear unambiguous bias towards 435 nm suggesting a plant absorption pigment in at this wavelength. In addition to Cytochromes, Phototropins considered an important group of photoreceptors in plants that regulate hypocotyl phototropism and chloroplast repositions under the influence of blue light to increase the efficiency of photosynthesis. This regulation is performed by NP1 and NPL1 Phototropins but only in the case of low blue light intensity (<1 μmol m\(^{-2}\) s\(^{-1}\)) (Sakai et al., 2001).

Within the Plant Factory, three lighting systems were compared with relatively small or subtle differences in the light supply, but none-the-less there were clearly significant differences between the three units. Two things were consistently revealed by the results, firstly: that a ratio of more blue over red increased growth and yield and secondly: that using blue light with a wavelength peak of 435 nm promoted growth up to 20% better than that of 450 nm. On the face of it, this is not surprising given that the measured plant absorption spectrum showed that the blue absorption peak is definitely at 435 nm and not at 450 nm as predicted by the McCree curve. This is a significant finding which could have implications for the future manufacture of red/blue grow-lights for Horticulture. Currently, all LED grow lights that are available for commercial use blue at 450 nm. A 20% yield increase for a shift of just 15 nm in the blue LED is remarkable and this finding needs to be cross-checked for other species.

Another significant finding was that the use a ratio of 1.6 blue to 1.0 red in the balance of LEDs in an array can also increase growth and yield in comparison to a 1:1 ratio B/R. This is also unexpected since most commercial horticultural grow-lights have a much higher red component than blue. It is difficult to understand why manufacturers of LED grow-lights have followed this pattern of manufacture. Possibly it is because most manufacturers are following the early market lead of companies like Phillips who assert that high red low blue LED lights are best for horticultural crops. It is possible that manufacturing costs have some part to play
here too, in that red LEDs are much cheaper to obtain than blue LEDs and are cheaper to run (use less electrical power). The approach to LED array design that was used in the current experiments was to go back to the plant light absorption spectrum and build an array that mirrored absorption i.e. higher blue than red and blue peak at 435 nm, and this approach has been definitely shown to be advantageous in plant performance under the Plant Factory conditions. This provides a significant body of evidence to the concept of “Designer Grow-Lights” that need to be optimised on a species-by-species basis. In agreement with the findings, a study showed that increasing the red light over the blue in the ratio of blue: red LEDs has a negative impact on FW. While a higher blue light over red in the blue: red ratio showed higher FW for Sweet Basil and five-fold energy efficiency in the high blue than Red ratio but the ratio of blue: red did not affect strawberries which indicates specific light recruitment for each plant species (Piovene et al., 2015).

**Conclusion**

In conclusion, each plant species has a specific light requirement. In the aspect of light quality, Sweet Basil can be cultivated more efficiently in a multi-tier hydroponic system under LEDs with a combination of blue light with a peak wavelength of 435 nm and red light with a peak wavelength of 663 nm. The combination of blue; red light is better when provided in a ratio of 1.6:1 to enhance Sweet Basil yield.
Chapter 5

General discussion
Growth and development of most plants, algae, and some bacteria species have evolved to depend on solar energy through the light controlled reaction of photosynthesis. Manipulating the physical aspect of this relationship through artificial lighting can give a greater understanding of the effect of light on plants. In the current research, light quality more than the other light regulating elements was of prime interest. Light quality has been widely studied over the last century but many of these studies have examined plant responses to the deviation in light quality. The general hypothesis of the study presented in this thesis is that plants have a specific requirement of light quality in order to gain maximum growth and quality. According to previously published research (Johnson *et al.*, 1999; Pettai *et al.*, 2005), the non-visible ranges of the spectrum have been found to have different effects on plant development but this was not investigated in the current study due to the lack of time. And this leaves investigating the outcomes of Far-Red, Infra-Red, and Ultra-Violet on the growth and quality of Sweet Basil in a controlled environment for future research. In the current study, only wavelengths in visible spectrum were investigated, especially, blue and red wavelengths. An investigation on the effect of green light with a peaked wavelength of 550 nm and yellow light with wavelength peaked at 596 nm showed the minimum effect that those wavelengths had on the growth, quality, and development of Basil (Figure 15). This finding co-relates with multiple recordings in the literature (Dougher & Bugbee, 2001; Foundation, 2018; Macedo *et al.*, 2011) that can confirm the minimum impact of additional Green and Yellow wavelengths but cannot be considered inessential or needless without vigorous analysis. The result of this analysis showed that Basil plants, in their preference to light source, leaned much more towards LED lighting units than conventional and industrial HPS lamps as a grow light. The initial light quality analysis showed the increase in yield, quality, and physiological properties of Basil when cultivated in a controlled environment using full artificial lighting in the form of an LED unit with specific narrowband spectrum. Comparing two LED lighting units, Basil thrived and
enhanced significantly under LED lighting unit that emits a combination of Blue light (459 nm) and Red (632 nm) more than a Wight emitting LED light unit that produced a mix of Blue light (451 nm), green light (550 nm), and Red light (620 nm). The essential oil was extracted from Sweet Basil to investigate the effect of light quality on the yield and composition on the volatile component of Basil. Following the trend of growth and physiological parameters ($A_{\text{max}}$, Gs, Fv/Fm, LA, FW, and DW), enhanced by the BR LEDs, higher volatile oil yield was obtained (Figure 23). The biosynthesis of most organic compounds was higher in plants under BR LEDs, mainly for Eucalyptol, Linalool, Eugenol, and Ethyl Cinnamate, leaving Estragole to be the exception. The highest accumulation rate was recorded in Linalool and lowest been Estragole. The abundance of Estragole was similar under the three lighting units (HPS, BR LED, and White LED). Although the Blue and Red light in both LED units had approximate wavelengths (only 8 nm for Blue light and 12 nm for Red light difference) yet the BR LED light showed better results. Additionally, the supplemental Green wavelength in the White LED unit combined with the difference in wavelengths for Blue and Red light from the BR LED unit seemed to stimulate the growth for Basil but not in the same rate. The enhancement of yield and quality resulted from the stimulation of Blue and Red light respecters at the same time which found to increase $A_{\text{max}}$ in plant species (Darko et al., 2014).

Building on the results from the initial analysis, emerged an important question; what specific spectrum wavelength that can enhance plants growth to the maximum level? There was no clear answer in the literature although it was granted yet questioned and revisited (Grow; Inada, 1976; McCree, 1972; Pocock; Ross & Sulev, 2000). The analysis was designed to gain more understanding of the specific spectrum requirements for plant development. A pigment protocol for Sweet Basil was followed and a clear answer was obtained yet needed more investigation and proofing. Sweet Basil showed a maximum absorption of light at three distinctive regions of the spectrum; an ultraviolet peak at 330 nm (shoulders from 300 to 360
nm), a broad Blue light peak at 435 nm (shoulders from 400 to 500 nm) and a Red light peak at 663 nm (shoulders from 640 to 690 nm) (Figure 27). The results of the absorption analysis, especially the shoulders of each region can explain the difference in commercial grow lighting specifications, for example, Osram and Philips use mostly Blue light in their LEDs with a peak wavelength of 450 nm (https://www.osram.com/), (https://www.lighting.philips.co.uk/home). The analysis showed as well a distinctive ratio of Blue to Red light that was calculated to (1.6) Blue to (1) Red. The final light quality analysis confirmed the results obtained from the absorption analysis. A tailored LED unit was applied as grow light for Sweet Basil with specific wavelengths in Blue (435 nm) combined with Red light (663 nm) with a ratio of B; R of 1.6; 1. The 435 nm Blue, 663 nm Red LED unit was compared with a commercially used LED unit with wavelengths of 450 nm Blue, 663 nm Red with the same ratio (1.6; 1). The fixing of the Red light at 663 nm in both units was designed to study the effects of two Blue light wavelengths only on the growth of Sweet Basil. A ratio combative LED unit was designed as well with wavelengths of 450 nm, 663 nm but with a ratio of 1:1 Blue to Red to gain more compression about the importance of Blue to Red ratio on the growth and development of Sweet Basil. The absorption analysis was a success when growth parameters and physiological responses of Sweet Basil was greater in plants grown under (Blue/Red 435 nm, 663 nm LED) unit compared with the commercial LED unit. The combination of maximum Amax and Gs under B435/R663 helped the plants to grow somehow uniformly taller, largely foliaged, and higher FW/DW with showing healthier indications (Fv/Fm). As expected the commercial LED unit with a ratio of Blue to Red is 1; 1 promoted the lowest growth indications which indicates the importance of the light combination ratio as well as the light quality. The LED analysis was designed in a closed environment and when compared with a glasshouse treatment, which depended on solar irradiation with supplementary HPS lighting, showed more robust yield than the glasshouse treatment.
The next step in the evolution of crop production is the cultivation of plants sustainably which includes switching from open-field farming to indoor growing. The main challenge concerning indoor cultivation of plant species (including Sweet Basil) is artificial lighting. Mastering both the production of useable light at low cost and defining the recipe for optimum lighting conditions will help the transition to perfecting the rest of the environmental elements, such as rooting substrates, needed by the plants. From this point, an investigation was designed to compare the efficiency of compost cultivation versus soilless cultures. Growing Sweet Basil in compost requires the need for constant regular watering and fertilizing when in contrast an alternative in the form of soilless culture can provide an easier solution. Furthermore, the results obtained from the initial analysis of cultivation methods showed the preferably of soilless cultivation (hydroponics) to soil-base growing. Hydroponic cultivation systems provide a great environment for plants to obtain moisture and nutrients easily but it can be found that this is affected by the type of media used in the system. Comparing three types of hydroponic mediums; clay pebble, Rockwool, and coco coir showed optimum growth and development in Rockwool. This resulted from the balance between retaining maximum moisture and the right aerated levels for the root system in Rockwool. Growth and development of Sweet Basil was not only found to be affected by hydroponic medium but also affected by the variation in cultivating methods. The investigation concluded that the hydroponic system was best for Sweet Basil cultivation in a closed environment compared to soil-based growing. Three systems were installed; Nutrient Film Technique, Raft/Floating system, and Ebb-Flow system. The data showed the preferably of maximum growth in the Raft/Floating system when compared with the other systems and Ebb-Flow showed the lowest growth parameters. Soil-based cultivation showed a low yield compared with hydroponic systems. This indicates the success and yield enhancement possibility of growing Sweet Basil in hydroponic systems consisting of Rockwool in a Raft/Floating system.
The final step in the current research is to combine all the pieces of the puzzle. A multi-tier hydroponic system was designed equipped with monitoring tools to regulate EC and pH and equipped with a dosing system that controlled the nutrient solution flow in the system. The system as a whole unit, called the Plant Factory, is fully controlled temperature and humidity wise. The Plant Factory was supplied with three units of artificial grow lights regulated by a timer to ensure the required photoperiod. Sweet Basil was cultivated in the Plant Factory using Rockwool and the data showed best growth and development was achieved in plants grown under a BR LED. The combination of optimum lighting (B435/R663), media (Rockwool), and cultivation method (Hydroponics) can provide a great recipe for maximum production of Sweet Basil around the year in plant factory establishment.

**Future Research**

Photosynthesis is a unique and fundamental reaction that require the perfection of several elements to achieve maximum efficiency. A limitation to one or more elements of this reaction would lead to photosynthetically stressed plants. Avoiding this stress was the main goal of the current research. However, only two important elements (light quality and growing methods) were studied in the present study. Much more analysis is required to attain wholesome optimization of the production process in a plant factory. Nutrient uptake and nutrient regulation are known to have effects on plant growth and quality and carbon dioxide concentration also affects growth and quality of Sweet Basil. Antioxidant activities in Sweet Basil requires more analysis in the context of plant factory production. Even the elements studied in this research require more analytical investigation for further variables such as the effects of a combination of Blue light 435 nm and Red light 663 nm on the volatile content of Sweet Basil and other pharmaceutical plant species. Similarly the growth regulating effects of UV-B and Far-Red light requires further investigation. Regulating the hydroponic solution can have a significant impact on the growth, quality, and chemical composition of essential oils of
Sweet Basil. A decrease in potassium to nitrogen ratio in nutrient solution had a positive effect on the FW of Berries as well as the increase in fragrance. Switching EC levels from high to normal (2 dS/m) at primary development stage resulted in high FW and increased flavour compared with the cultivation of Berries in regular EC level and a high level of EC led to decline in biomass. A decline in yield shown as well when increasing plants placements in a dense arrangement. It found that there is an interaction as well between calcium, nitrogen, potassium, and phosphorus, in a hydroponic system, and yield of Barley and Rocket, quality of Sweet Basil (Kiferle, Maggini & Pardossi, 2013; MacLeod, 1969; Santamaria, Elia & Serio, 2002; Sarooshi & Cresswell, 1994; Suh & Park, 2000). When managing hydroponic systems, this too must be taken into consideration, cultivation of Sweet Basil and a nutrient control system with built-in monitoring tools for EC, pH, and other elements can provide a more robust method of production.
Appendices

Appendix (A): Training courses attended

- BIO5131 Postgraduate Research Skills & Methods (17/AU/SB/M). Contained:
  Project Proposal and Literature Review. Level 7, 20 Credits. Passed (67.00%)
- BIO5125 Sustainable Use of Resources in Biological Systems (17/AU/SB/M).
  Contained: Report 1, 2 and a Seminar. Level 7, 20 Credits. Passed (65.50%).
- Molecular Biology module.
- Induction for new Research Students, 18 October 2017.
- Research Owning and Using, 6 November 2017.
- Presenting to an audience, 9 November 2017.
- Designing a poster, 15 November 2017.
- A session with information specialist Kim Davis on literature searching, 16 November 2017.
- Diversity in the Workplace, 2 October 2018.

Appendix (B): Outputs of the research

  Effect of light quality on the growth and biochemical compositions of two species of Basil (Ocimum basilicum & O. minimum).
- Aldarkazali M, McMulkin N, Rihan H, Fuller M. Analysis of three different mediums of Hydroponic systems for growing Sweet Basil (Ocimum basilicum). Poster presented at:
  The launching of the Plant Factory, 2018 August 14, Plymouth, University of Plymouth.
- University of Plymouth, 2018, ‘Sustainable Earth’, Plymouth, University of Plymouth, 28-29 June.
• Agri-Tech East, 2019, ‘Bringing the outside in - Innovating for Controlled Environment Agriculture’, Hertfordshire, Rothamsted research centre, 19 March.

Effects of light quality on growth and biochemical composition of two species of Basil (Ocimum basilicum & O. minimum)

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1. Summary
Basil is a plant that is economically, medicinally and culturally significant that is grown intensively in glasshouses and increasingly under controlled lighting. New artificial lighting methods have recently been developed and the light emitting diode (LED) is one of the most advanced methods that is increasingly being used. This project will investigate the effect of light (using LEDs) on the growth and yield of Basil in a plant factory system.

2. Objectives
The study has two main objectives:
- **Objective 1**: to study the effect of light on the chemical composition and growth of Sweet Basil (Ocimum basilicum) Bush Basil and (Ocimum minimum).
- **Objective 2**: to investigate the effect of light spectra on the quantity of some important pharmaceutically secondary metabolites of the two species of Basil.

3. Methods & Results
The absorption spectrum of Basil was determined by grinding leaves (obtained from a local store in Plymouth and produced at Vitacress Farms, West Sussex, UK) with acetone and then analysing the solution using a JENWAY 7315 spectrophotometer. Several absorption peaks were identified (Fig 3) but there is a mismatch between the absorption curve and the idealised plant photosynthetic response (Fig 4).

![Absorption Spectrum](image)

**Fig 3**: Absorption spectrum of Basil leaf pigments as measured by a spectrophotometer.

![LED Panel](image)

**Fig 1**: LED panel used to illuminate plants

![Sweet Basil](image)

**Fig 2**: Sweet Basil (Ocimum basilicum)

![Plant Factory System](image)

**Fig 5**: Plant factory system

![Bush Basil](image)

**Fig 6**: Bush Basil (Ocimum minimum)

4. Significance
The project will demonstrate best light conditions for plant growth and to save energy. The study will support commercial Basil production through optimizing growth conditions. Researchers in field of biological sciences will benefit from the project as it will provide important information on the reaction between plants and the physical environment.

![Photosynthetic Response](image)

**Fig 4**: Idealised plant photosynthetic response to light (McCree 1972)

5. Future work
Basil's seed germination and growth, under various light intensities and wavelengths will be applied using the following treatments:
- **Control**: HPS lamps to be used.
- **Mix of 435**: 663, 450:663 nm (1.6:1), as derived from Fig 3.
- Treatment based on the energy supplement by light's photon energy.

- A (1.6:1) 435: 663nm LEDs and adding UV-B 270-400 nm once in a single day for 72 hours.
- Treatments containing commercially used lighting LED systems.

The research will be conducted in a "Plant Factory" (Fig 5). The energy source that will operate the system will be provided by solar power.
Analysis of three different mediums of Hydroponic systems for growing Sweet Basil (Ocimum basilicum)

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Summary
Basil is a plant that is economically, medicinally and culturally significant that is grown intensively in glasshouses.

Soilless agriculture has a great share of development since the early history of man. Solution culture techniques was the main method to establish the specific needs of the plants that’s growing in a hydroponic system.

Material & Methods
The three mediums were used in small pots (5x8 cm) (randomly distributed) to be applied in a raft system hydroponic in three tubs (40x70x20 cm) filled with 25 letters of water added to it 25ml of (Hydro A/B 1L B’Cuzz Nutrients).

The system was monitored to check the PH and EC regularly till harvest.

Readings obtained from the LCI-SD showed almost unified trends. Photosynthetic rate was higher when plants cultivated in Rockwool hydroponic medium and lowest was in the control. Stomatal of CO₂ in Sweet Basil leaves was the lowest in Coco coir and the highest in Rockwool.

Objectives
The study has two main objectives:

• Objective 1: to study the effect of growing Sweet Basil (Ocimum basilicum) in an hydroponic system.
• Objective 2: to investigate the effect of three different hydroponic mediums (Clay pebbles, Rockwool, Coco coir) on the growth and characteristics of Sweet Basil (Ocimum basilicum).

Results & Future work
Between the three different hydroponic mediums there was a significant effect of the growing mediums on chlorophyll activates, plants height, Stomatal conductance, leaf area, number of leaves, fresh and dry weight of Sweet Basil plants had better growth in Rockwool medium than other hydroponic mediums and all above was lowest in the control and in the Coco coir.

Fresh weight of Sweet Basil was the highest in Rockwool and the lowest in Clay pebbles medium. Sweet Basil plants cultivated in Clay pebbles hydroponic medium gave the lowest value of Dry weight of water and Rockwool gave the highest value. Fm/Fv was the highest in Rockwool hydroponic medium and was the lowest in the clay pebbles. Next research step will be testing the mediums in a plant factory layout.
Study of Sweet Basil production in a plant factory farming
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Our research aims to answer three main questions:

Which LED lighting will be most suitable for use in a Plant Factory?
The optimal wavelength may vary dependent on plant species, stage of development and the growth conditions. First step to answer this question is to determine the wavelengths of light that are maximally absorbed by a variety of plant species at various stages in development and under different growth conditions.

Which hydroponic medium will be most suited for growth of O. basilicum in a Plant Factory?
A variety of hydroponic mediums are available with a variety of advantages and disadvantages. Our study aims to determine the most suitable medium for growth by measuring physiological properties of O. basilicum in three growth mediums in comparison to soil.

What is the effect of various lighting environments on the chemical composition of the essential oil of O. basilicum?
Five commercially extracted compounds found within the essential oil of O. basilicum will be analysed for their presence and quantity within the oil of O. basilicum grown in three different lighting conditions.

Investigating the relative absorbance of photosynthetic pigments at a range of wavelengths for a variety of plant species:

Investigating the growth and development of O. basilicum in three different hydroponic mediums:

Using Floating/Float hydroponic system, three different hydroponic mediums, Rockwool (RW), Coco Coir (CC) and Clay Pebbles (CP) were assessed. In comparison to control (C), these mediums were evaluated for O. basilicum growth and development (figure 4).

Using the ECO-10 Highly Portable Ambient Photobioreactor System (HPAS) four key physiological parameters were measured to determine the extent of growth and development in the four growth mediums (figure 1):
- RW promoted higher Chlorophyll Fluorescence Rate Fv/Fm.
- RW showed highest levels of stomatal conductance Gs.
- RW showed highest levels of leaf weight weight Wt.

Investigating the effect of three different lighting conditions on the abundance of five chemical compounds within O. basilicum:

O. basilicum was grown under three different lighting environments: high pressure sodium (HPS), Skyline 480 Watt LED lighting unit and Blue/Red Mars LED lighting unit. Using gas chromatography (GC), the presence and quantity of five key biochemical compounds within the essential oil will be determined from O. basilicum samples grown within these three lighting environments.

Optimisation of the extraction of the essential oil of O. basilicum for GC:
Dry plant material (2 g) was ground in a mortar and pestle with 10 mL of FISHER Scientific’s HPLC grade CH3OH (Heaven’s) ± 95%. The resultant mixture was then added to a steriled column with the addition of 90 mL of Heaven’s. The mixture then drained and the solution collected. A significant amount of used Heaven was evaporated using a BUCHI R-124 Rotary Evaporator System. A blowdown technique using thermal Techne® Sample Concentrator and BUCHI Hydrogen gas was applied to evaporate the rest of the Heaven from the solution. The essential oils of Sweet Basil were then isolated in a vial. The vial was weighed after the blowdown to calculate the quantity of the essential oil extracted.

The essential oil of Sweet Basil (0.033 g) was added to 10 mL Heaven to make a stock solution. A working solution was then prepared by adding 1 mL of this stock solution into 1 mL of Heaven. Each of the stock solutions of each chemical (5 chemicals) were added into the GC vial to give a total of 5 mL. Then 0.5 mL of Heaven was then added to the GC vial to make a final test solution of 1 mL, solution of 250 mg·mL-1.

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