EVALUATIONS OF GROWTH AND NUTRITIONAL PROFILE OF MICROGREENS OF DIFFERENT CROPS UNDER VARIOUS LEDs LIGHT SPECTRUMS

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EVALUATIONS OF GROWTH AND NUTRITIONAL PROFILE OF MICROGREENS OF DIFFERENT CROPS UNDER VARIOUS LEDs LIGHT SPECTRUMS

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My Beloved Family

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CERTIFICATE

*This is to certify that thesis entitled, "***EVALUATIONS OF GROWTH AND NUTRITIONAL PROFILE OF MICROGREENS OF DIFFERENT CROPS UNDER VARIOUS LEDs LIGHT SPECTRUMS***" submitted to the Department of Horticulture, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (M. Sc.)*** *in* ***HORTICULTURE****, embodies the result of a piece of bona fide research work carried out by* ***SWARNA MAHAJAN****, Registration No.* ***19-10168*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

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The Author

EVALUATIONS OF GROWTH AND NUTRITIONAL PROFILE OF MICROGREENS OF DIFFERENT CROPS UNDER VARIOUS LEDs LIGHT SPECTRUMS

ABSTRACT

This present study was conducted at an indoor structure on the 5th floor of Dr. Wazed Mia Research Centre, Sher-e-Bangla Agricultural University, Dhaka. During the period from November to December, 2020. The experiment comprised of single factors that is viz., Five different concentrations of LEDs light (White light L_1: 100; Red light L_2: 100; Blue light L_3: 100; Red: Blue light L_4: 70:30 and Red: Green: Blue light L_5: 70:10:20). Four different crops (C_1: Mustard, C_2: Lettuce, C_3: Radish, C_4: Broccoli) were used as microgreen crops. Results showed that the highest hypocotyl length of C_1, C_2, C_3 and C_4 were (10.53cm, 8.47cm, 15.23cm and 11.43cm) from the treatment of L_4C_1, L_4C_2, L_4C_3, and L_4C_4 whereas the lowest (7.67 cm, 5.53 cm, 11.2 cm and 7.73 cm) were found from the control treatment L_1C_1, L_1C_2 L_1C_3 and L_1C_4 respectively. Treatment of L_4C_1, L_4C_2, L_4C_3, and L_4C_4 registered the highest fresh weight of C_1, C_2, C_3 and C_4 (0.1, 0.1, 0.1 and 0.1kg) and yield (0.115, 0.110, 0.135 and .125kg). The treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 gave the highest SPAD value C_1 (38.2nm), C_2 (16.9nm), C_3 (55.3nm) and C_4 (49.9nm), nitrogen (%) of C_1 (38.23%), C_2 (16.93%), C_3 (55.27%) and C_4 (93.93%), potassium (%) of C_1(0.19%), C_2(0.19%), C_3(0.22%) and C_4(0.16%) and antioxidant capacity of C_1 (0.22%), C_2 (0.23%), C_3 (0.19%) and C_4 (0.18%). The highest and lowest gross income was obtained from L_4C_1, L_4C_2, L_4C_3, and L_4C_4 treatment and L_1C_1, L_1C_2 L_1C_3 and L_1C_4 treatment. The highest (4.1, 3.9, 4.9 and 4.5) and lowest (2.6, 2.4, 2.7 and 2.6) benefit cost ratio was noted for L_4C_1, L_4C_2, L_4C_3, and L_4C_4 treatment and L_5C_1, L_5C_2 L_5C_3 and L_5C_4 treatment. So, Red and Blue (70:30) light can be economically used.

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ABBREVIATION AND ACRONYMS

AEZ	=	Agro-Ecological Zone
BBS	=	Bangladesh Bureau of Statistics
BCSRI	=	Bangladesh Council of Scientific Research Institute
cm	=	Centimetre
CV %	=	Percent Coefficient of Variation
DMRT	=	Duncan‘s Multiple Range Test
et al.,	=	And others
e.g.	=	exempli gratia (L), for example
etc.	=	Etcetera
FAO	=	Food and Agricultural Organization
g	=	Gram (s)
i.e.	=	id est (L), that is
Kg	=	Kilogram (s)
LED	=	Light Emitting Diode
LSD	=	Least Significant Difference
m^2	=	Meter squares
ml	=	Millilitre
M.S.	=	Master of Science
No.	=	Number
PPFD	=	Photosynthetic Photon Flux Density
SAU	=	Sher-e-Bangla Agricultural University
var.	=	Variety
^oC	=	Degree Celsius
%	=	Percentage
NaOH	=	Sodium hydroxide
GM	=	Geometric mean

mg	=	Milligram
P	=	Phosphorus
K	=	Potassium
Ca	=	Calcium
L	=	Litre
μg	=	Microgram
USA	=	United States of America
WHO	=	World Health Organization

CHAPTER I

INTRODUCTION

Microgreens are a category of edible salad crops that appearing in many upscale markets and restaurants. They are seedlings of vegetables and herbs consumed with tender cotyledons and the first pairs of leaves more or less developed. At harvest, plant height is from 2.5 to 8 cm depending on the species. They are harvested at the base of the hypocotyl when the first true leaves start to emerge (Xiao, 2012). The consumption of microgreens has increased, as a rich-nutrient crop with a high level of nutrition components concentration contains; vitamins, minerals, and antioxidants compared to mature greens, which are helpful in filling the nutritional gap challenges (Burlingame, 2014).

Microgreens can provide a high concentration of health-promoting phytochemicals. Specifically, microgreens of the family Brassicaceae, Asteraceae and Fabaceae have become a popular choice due to its easy way for germination and short growth length and providing wide flavours and colours. Thus, brassica microgreens are considered as a functional food, which serves as a health-promoting or disease preventing supplemental (Yorio, 2001).

Light emitting diodes (LEDs) is a new light source technology used for greenhouses facilities and space-limited plant growth chambers Recently, many studies demonstrated the influence of LEDs (blue or/and red) lighting on the plant vegetative parameters we explore the impact of different four LEDs lighting ratio (Red, Blue, and Green) on 10 Brassica 2 other family microgreens growth and nutritional profile (Ascorbic acid and antioxidant) (Brazaityte, 2016).

Light is one of the most important environmental factors regulating plant growth, development, and photosynthesis (Claypool and Lieth, 2020). Lighting-emitting diodes (LEDs) are regarded as the most effective light source with the highest potential and are being developed to provide powerful, effective, and environmental emission spectra covering the entire photo synthetically active radiation range to precisely regulate numerous types of light combinations (Avercheva *et al*., 2016). Light quality has more complex impacts on plant morphology and metabolism than light intensity or photoperiod (Chen *et al*., 2017).

Microgreens were grown under four different LEDs ratios (%); red: blue 80: 20 and 20: 80 (R_{80}: B_{20} and R_{20}: B_{80}), or red: green: blue 70: 10: 20 and 20: 10: 70 (R_{70}: G_{10}: B_{20} and R_{20}:G_{10}: B_{70}). Results indicated that supplemental lighting with green LEDs enhanced vegetative growth and morphology, while blue LEDs increased the mineral and vitamin contents. Interestingly, by linking the nutritional content with the growth yield to define the optimal

LEDs setup, it was found that the best lighting to promote the microgreen growth was the green LEDs combination (R_{70}: G_{10}: B_{20}) (Khaled, 2020).

Red-blue (RB) LED lighting systems are widely used for plant cultivation because red and blue light are effectively absorbed by photosynthetic pigments (Phansurin, 2017**).** The use of LED lighting to enhance productivity and in indigenous vegetable microgreen cultivation (Harakotr-2019).

Red light results in the highest quantum yield of CO_2 fixation among the wavelengths in the photosynthetically active spectrum (Hogewoning *et al*., 2012; Wu *et al*., 2019). It has been reported that blue-light signaling triggers processes such as photomorphogenesis, stomatal opening, and phototropism, which broadly affect the level of photosynthesis (Horrer *et al*., 2016; Huche-Thelier *et al*., 2016). Blue light enhances the accumulation of carotenoids, flavonoids, and anthocyanins without substantially affecting plant morpho-anatomical traits (Landi *et al*., 2020; Zhang *et al*., 2020), but long-term blue light exposure may affect plant growth and morphology (Huche- Thelier *et al*., 2016). However, red light strongly alters morphology and physiology without showing positive effects on secondary metabolites (Zhang *et al*., 2020). Some studies have verified the importance of the combination of red and blue light for improving plant growth and nutritional quality compared with than monochromatic light in crops such as lettuce, cucumber, soybean seedlings, and pakchoi (Chen and Yang, 2018; Song *et al*., 2020).

One significant benefit of using LEDs is the ability to select light qualities that have beneficial impacts on plant morphology and phytochemical content of brassica (Brassica sp.) microgreens (Craver, 2017).

Plants have varied morphological and physiological responses to specific light spectrum, and the current advancement of LEDs enables one to tailor the spectrum to obtain favorable plant growth or nutritional values (Mickens *et al*., 2018).

With conceiving the above scheme in mind, the present research work has been undertaken in order to fulfilling the following objectives:

1. To develop microgreens in a controlled grow-house under LEDs light spectrum.

2. To investigate the growth and yield of microgreens crops under different coloured light.

3. To find out the nutritional constituent of the microgreens of different crops under different LEDs light spectrums.

CHAPTER II

REVIEW OF LITERATURE

Development of plant growth and yield using different LED-light spectrum is a new idea of vegetable farming. Among different LED-light spectrum treatments, microgreen has been grown to find out the best option using LED-light spectrum. Very limited studies have been performed in this aspect. Some of the recent past information on the development of growth and nutrient content of different microgreen crops under different LED-light spectrum in vertical farming have been presented (alphabetically) in this chapter.

Ausra Brazaityte *et al*., (2016) state that Supplemental 520-and 622-nm lighting was more efficient for nitrate reduction, while the anti-oxidative system indices were enhanced by 595-nm diodes. Supplemental 366-and 390-nm UV-radiations have been more favourable for antioxidant accumulation. Short-term (3-days before harvesting) lighting with high PPFD level of red (638 nm) LEDs increased the amounts of the secondary metabolites of micro greens under both cultivation conditions.

Ausra Brazaityte *et al*., (2018) state that an increase of various mineral elements content was mostly caused by higher percentage of blue light.

Ausra Brazaityte *et al*., (2019) stated Ultraviolet A (UV-A) light-emitting diodes (LEDs) could serve as an effective tool for improving the content of health-promoting bioactive compounds in plants in controlled-environment agriculture (CEA) systems. The goal of this study was to investigate the effects of UV-A LEDs at different wavelengths (366, 390, and 402 nm) and durations (10 and 16 h) on the growth and phytochemical contents of mustard microgreens (*Brassica juncea* L. cv. “Red Lion”), when used as supplemental light to the main LED lighting system (with peak wavelengths of 447, 638, 665, and 731 nm). Plants were grown for 10 days under a total photon flux density (TPFD) of 300 μmol m^{-2} s^{-1} and 16-h light/8-h dark period. Different UV-A wavelengths and irradiance durations had varied effects on mustard microgreens. Supplemental UV-A radiation did not affect biomass accumulation; however, the longest UV-A wavelength (402 nm) increased the leaf area of mustard microgreens, regardless of the duration of irradiance. The concentration of the total phenolic content and α-tocopherol mostly increased under 402-nm UV-A, while that of nitrates increased under 366- and 390-nm UV-A at both radiance durations. The contents of lutein/zeaxanthin and β-carotene increased in response to the shortest UV-A wavelength (366 nm) at 10-h irradiance as well as longer UV-A wavelength (390 nm) at 16 h irradiance. The most positive effect on the accumulation of mineral elements, except iron, was observed under longer UV-A wavelengths at 16-h irradiance. Overall, these results suggest that properly composed UV-A LED parameters in LED lighting systems could improve the nutritional quality of mustard microgreens, without causing any adverse effects on plant growth.

Barbara B. *et al*., (2014) state that Recent microgreens include initiative on biodiversity for food and nutrition, sustainable diets, and sustainable consumption and production. As new as it all seems, the consumption of microgreens has increased, as a rich-nutrient crop with a high level of nutrition components concentration contains; vitamins, minerals, and antioxidants compared to mature greens.

Bhornchai Harakotr *et al*., (2019) state that Light irradiance levels of 330, 220, and 110 $\mu mol.m^{-2}. S^{-1}$ photosynthetically active flux density (PPFD) was compared, with fluorescence lighting as control. Irradiance at 330 $\mu mol.\ m^{-2}. S^{-1}$ PPFD was found to be optimal for growth and accumulation of bioactive compounds by water convolvulus, red holy basil, dill, and lemon basil micro greens, producing the greatest dry weight, total phenolic and flavonoid content, and ABTS and DPPH free radical scavenging.

Chen *et al*., (2017) state that however, under alternating red and blue light with intervals of 2 and 4 h, soluble sugar and ascorbic acid levels were significantly increased, but the nitrate content was decreased.

Chen *et al*., (2019) state that Light is central for the evolution and sustainability of life on our planet. For plants, light can be a source of energy and an environmental signal. Plants harness light energy from the sun to convert carbon dioxide and water into carbohydrates and release oxygen into the atmosphere. Plants have also evolved many types of photoreceptors to perceive different light qualities, such as wavelength, intensity and duration, to regulate a broad range of developmental and physiological processes. In a study of different intervals of alternating red and blue light, treatment with an interval of 1 h was shown to be beneficial for the accumulation of biomass, sucrose, and starch in lettuce and promoted electric efficiency and light use efficiency.

Ellen R. Turner *et al*., (2020) stated microgreens have gained increasing popularity as food ingredients in recent years because of their high nutritional value and diverse sensorial characteristics. Microgreens are edible seedlings including vegetables and herbs, which have been used, primarily in the restaurant industry, to embellish cuisine since 1996. The rapidly growing microgreen industry faces many challenges. Microgreens share many characteristics with sprouts, and while they have not been associated with any foodborne illness outbreaks, they have recently been the subject of seven recalls. Thus, the potential to carry foodborne pathogens is there, and steps can and should be taken during production to reduce the likelihood of such incidents. One major limitation to the growth of the microgreen industry is the rapid quality deterioration that occurs soon after harvest, which keeps prices high and restricts commerce to local sales. Once harvested, microgreens easily dehydrate, wilt, decay and rapidly lose certain nutrients. Research has explored preharvest and postharvest interventions, such as calcium treatments, modified atmosphere packaging, temperature control, and light, to maintain quality, augment nutritional value, and extend shelf life.

Gene E. Lester *et al*., (2012) stated that different microgreens provided extremely varying amounts of vitamins and carotenoids. Total ascorbic acid contents ranged from 20.4 to147.0 mg per 100 g fresh weight (FW), while β-carotene, lutein/zeaxanthin, and violaxanthin concentrations ranged from 0.6 to 12.1, 1.3 to 10.1, and 0.9 to 7.7 mg/100 g FW, respectively. Phylloquinone level varied from 0.6 to 4.1 μg/g FW; meanwhile, α-tocopherol and γ- tocopherol ranged from 4.9 to 87.4 and 3.0 to 39.4 mg/100 g FW, respectively. Among the 25 microgreens assayed, red cabbage, cilantro, garnet amaranth, and green daikon radish had the highest concentrations of ascorbic acids, carotenoids, phylloquinone, and tocopherols, respectively. In comparison with nutritional concentrations in mature leaves (USDA National Nutrient Database), the microgreen cotyledon leaves possessed higher nutritional densities.

Giedre Samuoliene *et al*., (2012) stated the impact of supplementary short-term red LEDs lighting on the antioxidant properties of microgreens. Different species of red and green leaf microgreens (amaranth, basil, mustard, spinach, broccoli, borage, beet, kale, parsley, pea) were grown to harvest time in a greenhouse in a peat substrate under daylight with supplementary lighting provided by standard high-pressure sodium lamps (HPS). At pre-harvest stage of 3 days, HPS lamps were supplemented by 638 nm LEDs, whereas reference plants continued staying under lighting conditions identical to those of growth. PPFD generated by illuminator was 170 µmol m^{-2} s^{-1} and net PPFD generated by the illuminator in combination with HPS lamps - 300 µmol m^{-2} s^{-1} (16-h; 19-22/15-18°C). Due to the increased activity of the metabolic system for the protection from properties of microgreens were changed. Natural antioxidant compounds were in order: pea> broccoli> borage> mustard> amaranth> basil> kale> beet> parsley> tatsoi. Total phenols concentration increased with supplemental red in all microgreens from 9.1% in mustard to 40.8% in tatsoi, except of amaranth, where decrease of 14.8% was observed. Ascorbic acid content increased in amaranth (79.5%), pea (65.2%), kale (60.6%), broccoli (59.1%) and mustard (25.0%), but decreased in basil (53.9%) and borage (46.9%), and had no significant effect in tatsoi, beet and parsley. Total anthocyanins significant increase in broccoli (45.1%), kale (44.0%), amaranth (38.0%), tatsoi (34.5%), parsley (27.0%) and pea (14.6%), significant decrease was detected in borage (51.8%), mustard (45.1%) and beet (43.3%) and was not significantly affected in basil.

Giedre Samuoliene *et al*., (2013) state that A system of five lighting modules with 455, 638, 665 and 731 nm LEDs at a total photosynthetic photon flux densities (PPFD) of 545, 440, 330, 220 and 110 µmol m−2s−1 respectively were used. Insufficient levels of photosynthetically active photon flux (110 μmol m−2 s−1) suppressed normal growth and diminished the nutritional value of the Brassica microgreens studied.

Havaux, (1998) stated that due to the chemical structures of chlorophyll a and b, the absorption 8 spectra are not uniform across PAR and they have minimal absorption in the 500–600 nm range, thus, reflecting the colours of light green and turquoise, respectively. In some plants, accessory pigments, such as carotenoids (carotenes and xanthophylls), are produced to help absorb light in the blue green spectrum to enhance photosynthesis.

Hogewoning *et al*., (2010), Johkan *et al*., (2010) and Matsuda *et al.*, (2007) stated that BL is also known to increase the chlorophyll content.

Hogewoning *et al*., (2010) and Matsuda *et al*., (2007) suggested that a greater fraction of BL is associated with the development of leafy characterized by a high leaf thickness and photosynthetic capacity. (Inskeep and Bloom, 1985) stated the approximate absorption maxima of chlorophyll a are at 430 nm and 662 nm and those of chlorophyll b are at 453 and 642 nm.

Joshua K. Craver *et al*., (2017) stated that A daily light integral (DLI) of 6, 12, or 18 $mol{\cdot}m^{-2}{\cdot}d^{-1}$ was achieved from SS LED arrays with light ratios (percent) of red: blue 87:13 (R_{87}:B_{13}), red: far- red: blue 84:7:9 (R_{84}:FR_7:B_9), or red: green: blue 74:18:8 (R_{74}:G_{18}:B_8) with a total photon flux from 400 to 800 nm of 105, 210, or 315 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ for 16 hours, respectively. Light quality affected total integrated chlorophyll with higher values observed under the light ratio of R_{87}:B_{13}compared with R_{84}: FR_7: B_9 and R_{74}: G_{18}: B_8 for kohlrabi and mustard microgreens, respectively. For kohlrabi, with increasing light intensities, the total concentration of anthocyanins was greater compared with those grown under lower light intensities. In addition, for kohlrabi, the light ratios of R_{87}: B_{13} or R_{84}: FR_7: B_9 produced significantly higher anthocyanin concentrations compared with the light ratio of R_{74}: G_{18}: B_8 under a light intensity of 315 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$.

Khaled Y. Kamal, *et al*., (2020) stated that supplemental lighting with green LEDs (R_{70}: G_{10}: B_{20}) enhanced vegetative growth and morphology, while blue LEDs (R_{20}: B_{80}) increased the mineral and vitamin contents. Interestingly, by linking the nutritional content with the growth yield to define the optimal LEDs setup, we found that the best lighting to promote the microgreen growth was the green LEDs combination (R_{70}: G_{10}: B_{20}). Remarkably, under the green LEDs combination (R_{70}: G_{10}: B_{20}) conditions, the microgreens of Kohlrabi purple, Cabbage red, Broccoli, Kale Tucsan, Komatsuna red, Tatsoi and Cabbage green, which can benefit human health in conditions with limited food, had the highest growth and nutritional content.

Kopsell A. *et al*., (2013) stated that the impact of short-duration blue light on phytochemical compounds, which impart the nutritional quality of sprouting broccoli microgreens. Broccoli microgreens were grown in a controlled environment under LEDs using growing pads. Seeds were cultured on the pads submerged in deionized water and grown under a 24-hour photoperiod using red (627 nm)/blue (470 nm) LEDs (350 mmol/mL^2s L1) at an air temperature of 23°C. On emergence of the first true leaf, a complete nutrient solution with 42 mgLL1 of nitrogen (N) was used to submerge the growing pads. At 13 days after sowing, broccoli plantlets were grown under either: 1) red and blue LED light (350 mmol/mL^2sL1); or 2) blue LED light (41 mmol/mL^2sL1) treatments for 5 days before harvest. The experiment was repeated three times. Frozen shoot tissues were freeze-dried and measured for carotenoids, chlorophylls, glucosinolates, and mineral elements. Comparing the two LED light treatments revealed the short duration blue LED treatment before harvest significantly increased shoot tissue b-carotene (P £ 0.05), violaxanthin (P £ 0.01), total xanthophyll cycle pigments (P £ 0.05), glucoraphanin (P £ 0.05), epiprogoitrin (P £ 0.05), aliphatic glucosinolates (P £ 0.05), essential micronutrients of copper (Cu) (P = 0.02), iron (Fe) (P £ 0.01), boron (B), manganese (Mn), molybdenum (Mo), sodium (Na), zinc (Zn) (P £ 0.001), and the essential macronutrients of calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), and sulfur (S) (P £ 0.001). Results demonstrate management of LED lighting technology through preharvest, short-duration blue light acted to increase important phytochemical compounds influencing the nutritional value of broccoli microgreens.

Li and Kubota *et al*., (2009) and Metallo *et al*., (2018) said that BL also regulates several plant morphogenic responses including leaf expansion and shoot elongation.

Masahumi *et al*., (2010) stated that at the end of the light treatment, that is 17 days after sowing (DAS), the leaf area and shoot fresh weight (FW) of the lettuce seedlings treated with red light increased by 33% and 25%, respectively, and the dry weight of the shoots and roots of the lettuce seedlings treated with blue-containing LED lights increased by greater than 29% and greater than 83% compared with seedlings grown under a white fluorescent lamp (FL). The shoot/root ratio and specific leaf area of plants irradiated with blue-containing LED lights decreased. At 45 DAS, higher leaf areas and FWs were obtained in lettuce plants treated with blue-containing LED lights. The total chlorophyll (Chol 1) contents in lettuce plants treated with blue-containing and red lights were less than that of lettuce plants treated with FL, but the Chol a/b ratio and carotenoid content increased under blue-containing LED lights. Polyphenol contents and the total antioxidant status (TAS) were greater in lettuce seedlings treated with blue-containing LED lights than in those treated with FL at 17 DAS. The higher polyphenol contents and TAS in lettuce seedlings at 17 DAS decreased in lettuce plants at 45 DAS. In conclusion, our results indicate that raising seedlings treated with blue light promoted the growth of lettuce plants after transplanting. This is likely because of high shoot and root biomasses, a high content of photosynthetic pigments, and high antioxidant activities in the lettuce seedlings before transplanting. The compact morphology of lettuce seedlings treated with blue LED light would be also useful for transplanting.

Myung-Min Oh *et al*., (2009) stated that certain phenolic compounds can be induced in lettuce by environmental stresses. Of all the stress treatments, high light produced the greatest accumulation of phenolic compounds, especially following the stress treatments during the recovery. In addition, key genes such as phenylalanine ammonia-lyase (PAL), L-galactose dehydrogenase (L-GalDH), and g-tocopherol methyltransferase (g-TMT) involved in the biosynthesis of phenolic compounds, ascorbic acid, and a-tocopherol, respectively, were rapidly activated by chilling stress while heat shock and high light did not appear to have an effect on the expression of PAL and g-TMT. However, L-GalDH was consistently activated in response to all the stresses. The results also show that these mild environmental stresses had no adverse effects on the overall growth of lettuce, suggesting that it is possible to use mild environmental stresses to successfully improve the phytochemical content and hence the health-promoting quality of lettuce with little or no adverse effect on its growth or yield.

Natiely Gallo De La Paz *et al*., (2022) stated that with the growing demand for natural, healthy, minimally processed products, reducing environmental damage and increasing production worldwide, a range of opportunities opens up for the field of closed systems (factory plan) for food production where production can be controlled throughout the year. All these have a goal of ending malnutrition, reducing chronic diseases, and having products with a high content of bioactive compounds. Plant factory is a closed food production system that allows to produce food products all year-round in small spaces and with high yields, this is the case of microgreens (they can be defined as seedlings with developed cotyledons as well as their first true leaves and are harvested between 7 and 20 days after sowing in function of the species). To achieve this, it is necessary to find the best growing conditions in a closed system, such as humidity, temperature, nutrients, planting density, and LED light, choosing the specific spectral characteristics for each crop.

Neil C. Y. *et al*., (2001) state that Radish (*Raphanus sativus* L. cv. Cherriette), lettuce (*Lactuca sativa* L.cv. Waldmann's Green), and spinach (*Spinacea oleracea* L. cv. Nordic IV) plants were grown under 660-nm red light –emitting diodes (LEDs) and were compared at equal photosynthetic photon flux (PPF) with either plants grown under cool- white fluorescent lamps (CWF) or red LEDs supplemented with 10% (30 mol. $m^{-2}s^{-1}$) blue light (400-500nm) from blue fluorescent (BF) lamps.

Netto *et al*., (2005) stated that the chlorophyll content is determined by mainly two methods, which are the absorption of light of isolated chlorophyll in aqueous acetone and the measurement of leaf reflectance and transmission level using a Soil Plant Analysis Development (SPAD) chlorophyll meter.

Qian Li *et al*., (2009) stated that using UV-A, blue (B), green (G), red (R), and far-red (FR) light-emitting diodes (LEDs), we investigated the effects of different supplemental light qualities on phytochemicals and growth of 'Red Cross' baby leaf lettuce (*Lactuca sativa* L.) grown at a high planting density under white fluorescent lamps as the main light source inside a growth chamber. Photon flux added by supplemental LEDs for UV-A, B, G, R and FR were 18, 130, 130, 130 and 160μ molm^{-2} s^{-1}, respectively. Photosynthetic photon flux (PPF, 400–700 nm), photoperiod, and air temperature (day/night) was 300 μ molm^{-2} s^{-1}, 16 h, and 25 °C/20 °C in all treatments including white light control. After 12 days of light quality treatment (22 days after germination), phytochemical concentration and growth of lettuce plants were significant affected by light treatments. Anthocyanins concentration increased by 11% and 31% with supplemental UV-A and B, respectively, carotenoids concentration increased by 12% with supplemental B, phenolics concentration increased by 6% with supplemental R while supplemental FR decreased anthocyanins, carotenoids and chlorophyll concentration by 40%, 11% and 14%, respectively, compared to those in the white light control. The fresh weight, dry weight, stem length, leaf length and leaf width significantly increased by 28%, 15%, 14%, 44% and 15%, respectively, with supplemental FR light compare to white light, presumably due to enhanced light interception by enlarged leaf area under supplemental FR light. Although the mechanisms of changes in phytochemicals under different supplemental light quality are not well known, the results demonstrated that supplemental light quality could be strategically used to enhance nutritional value and growth of baby leaf lettuce grown under white light.

Qinglu Ying *et al*., (2020) stated that to optimize blue light proportion in red and blue light-emitting diode (RB-LED) lighting for microgreen production, the yield and appearance quality of cabbage, kale, arugula and mustard were investigated under RBLED lightings with 5%, 10%, 15%, 20%, 25% and 30% blue light. For each lighting treatment, the total photosynthetic photon flux density was set at ≈ 300 μmolm^{-2} s^{-1}, and the air temperature during light/dark was set at≈21/ 17 °C. As a result, neither fresh nor dry yield was affected by blue light percentage for the tested species except cabbage, which showed quadratic (peaking at 15%) responses in crop yield. Hypocotyl length and cotyledon area of kale and mustard decreased linearly with increasing blue light percentage which, however, did not affect arugula or cabbage in these two traits. For plant colour, cotyledons were darker red for mustard and less pure green for the other three species under higher blue light percentage. This was indicated by a negative linear response of hue angle or green chromaticity to increasing blue light percentage. These findings suggested that responses to blue light percentage varied with plant traits and microgreen species. To reach a balance of yield and appearance quality, 15% blue light was recommended for indoor production of cabbage microgreens, while 5% blue light for the other three species, under similar environmental conditions.

Roberta B. *et al*., (2017) stated that Microgreens are gaining interest for claimed high nutraceutical properties, but data on their chemical composition are so far limited. Although often grown hydroponically, their mineral requirements are still unknown. This study aimed to provide an insight into yield, mineral uptake, and quality of basil, Swiss chard, and rocket microgreens grown in a hydroponic system. With reference to data reported in literature for the same species hydroponically grown but harvested at adult stage, these microgreens yielded about half, with lower dry matter percentage, but higher shoot/root ratio. They showed high concentrations of some minerals, but their nutrient uptake was limited due to low yield.

Shimokawa *et al*., (2014) stated there are two lighting methods for exposing plants to red and blue light: the familiar method of simultaneous lighting and the Shigeo Method, the core concept of which is the alternation of red and blue light irradiation.

Shimokawa *et al*., (2014) stated Alternating red and blue light was shown to significantly enhance lettuce growth when the total intensity was the same as that under the simultaneous irradiation with red and blue light each day.

Specht *et al*., (2014) stated that Vertical grow-house technology does not require huge arable land to produce crops and thus is agriculturally independent. This innovation utilizes the horizontal and vertical spaces 6 more effectively, thereby, producing higher yield per unit volume under controlled environmental conditions of temperature, light, carbon dioxide and humidity. There are different types of vertical grow-house innovations like hydroponics, aeroponics, and aquaponics where the nutrients are effectively utilized and monitored for physical and chemical parameters like quality, pH, and solubility in water. Since vertical grow -house is experimented within a closed and controlled environment, sunlight as a source of light for carrying out photosynthesis is replaced by artificial lights with different spectra and intensities. In such a case, LED lights are more effective with high energy use efficiency and durability than traditional light sources like fluorescent lamps.

Stefania Toscano *et al*., (2021) stated the response to light spectra was often species-specific, and the interaction effects were significant. Morpho biometric parameters were influenced by species, light, and their interaction; at harvest, in both species, the fresh weight was significantly greater under B. In amaranth, Chol a was maximized in B, whereas it did not change with light in turnip greens. Sugar content varied with the species but not with the light spectra. Nitrate content of shoots greatly varied with the species; in amaranth, more nitrates were measured in R, while no difference in turnip greens was registered for the light spectrum effect. Polyphenols were maximized under B in both species, while R depressed the polyphenol content in amaranth.

T G Shibaeva *et al*., (2022) stated the effect of continuous lighting applied in the end-of-production period on growth and nutritional quality of radish (*Raphanus sativus* var. radicula), broccoli (*Brassica oleracea* var. italic), mizuna (*Brassica rapa* var. nipposinica) and arugula (*Eruca sativa*) was investigated in growth chambers under LED lighting. Microgreens were grown under 16 h photoperiod and 3 days before harvest half of plants were placed under continuous lighting conditions. Pre-harvest continuous lighting treatment increased yield, robustness index, and shorten time to harvest in radish, broccoli, mizuna and arugula microgreens. The end-of-production treatment has also led to higher content of compounds with antioxidative properties (flavonoids, proline) and increased the activity of antioxidant enzymes (CAT, APX, GPX) by inducing mild photooxidative stress. Increased antioxidative status added nutritional value to microgreens that can be used as functional foods providing health benefits. Pre-harvest treatment by continuous lighting is suggested as the practice than can allow producers to increase yield, aesthetic appeal, nutritional quality, and market value of Brassicacea microgreens.

Teodor RUSU *et al*., (2021) stated the Lettuce microgreens contain higher quantities of phytonutrients and minerals and lower quantities of nitrates at the early stage of development than at the completely developed stage. The environmental conditions that influence the development of lettuce microgreens (and their quality) in a hydroponic system are as follows (average ideal values): light (400 W), photoperiodicity (12 h), light intensity (400 μmol m^{-2} s^{-1}), colour spectrum (440-460 nm), temperature (20 ± 2 °C), and humidity (80 ± 5 %). The nutritional solution in a hydroponic system must be carefully monitored, by checking certain essential parameters such as the following (average ideal values): pH (6.3 ± 0.4), electrical conductivity (1.8 ± 0.2 mS), dissolved oxygen (6 mg L^{-1}), and temperature (18 ± 2 °C). The analysis of expert literature reveals that there is a need to establish certain protocols for cultivating microgreens in hydroponic systems, to minimize the factors that can negatively influence the plants, in order to obtain higher concentrations of active substances.

Terashima *et al*., (2009) stated that there is a significant loss of BL energy resulting from the absorption by non-photosynthetic pigments, including anthocyanin and accessory photosynthetic pigments that have inefficient energy transfer to chlorophyll. Azad *et al*., (2020), Hogewoning *et al*., (2010), Lee *et al*., (2016) and Yang *et al*., (2016) stated that RL induces many physiological responses including leaf development, stomatal opening, chlorophyll and carbohydrate accumulations.

Uyory Choe *et al.*, (2018) stated that consumption of vegetables can significantly reduce the risk of many chronic diseases. Dietary guidelines for 2015–2020 from the U.S. Department of Agriculture and the U.S. Department of Health and Human Services recommend 1–4 cups of vegetables per day for males and 1–3 cups of vegetables per day for females, depending on their age. However, the average intake of vegetables is below the recommended levels. Microgreens are young vegetable greens. Although they are small, microgreens have delicate textures, distinctive flavors, and various nutrients. In general, microgreens contain greater amounts of nutrients and health-promoting micronutrients than their mature counterparts. Because microgreens are rich in nutrients, smaller amounts may provide similar nutritional effects compared to larger quantities of mature vegetables. However, literature on microgreens remains limited. In this Review, we discuss chemical compositions, growing conditions, and biological efficacies of microgreens. We seek to stimulate interest in further study of microgreens as a promising dietary component for potential use in diet-based disease prevention.

Vastakaite V. *et al.*, (2016) state that Mustard micro greens grown under supplemental 470-, 505-, 590-, 627 nm LEDs accumulated significantly ($P<0.05$) higher contents of Mn, Mg, Fe, Zn, Ba. All supplemented LED wavelengths had influence on enhanced contents of Ca, Mg, Cu, P, S, Ba, Sr in basil micro greens.

Viktorija V. *et al.*, (2015) stated the effect of industrially designed light-emitting diode (LED) lamp lighting on the nutritional quality of Brassicaceae microgreens. Red pak choi (*Brassica rapa* var. chinensis 'Rubi F1'), tatsoi (*Brassica rapa* var. rosularis) and mustard (*Brassica juncea* L. 'Red Lion') were grown in a greenhouse (20±2/18±2 °C) during winter season, and the solar daily integral (DLI) was ~3.46±1.16 mol m^{-2} d^{-1}. The light spectra of lamp consist of 8 violet (420-430), 16 blue (460-470 nm), 8 orange (610-615 nm), 3 red (620-630 nm), 56 red (660-670 nm), 8 white (contain blue (400-500 nm), green (500-600 nm) and red (600-700 nm)) LEDs. The treatments of ~150 and ~250 μmol m^{-2} s^{-1} LED irradiance levels (LED 150 and LED 250) for 16 h d^{-1} in comparison with high pressure sodium (HPS) lamps (~150 μmol m^{-2} s^{-1}) as a control were performed. Photo physiological response to the artificial light varied among Brassicaceae species. Microgreens treated with LED 150 and LED 250 were significantly ($P\leq0.05$) shorter and formed smaller hypocotyls. The photooxidative changes were evoked by both lighting treatments and led to higher phytochemical (phenols, ascorbic acid, flavanol's, anthocyanins and mineral element (Ca, K, Mg, Na, P, Fe, Zn) contents, and the DPPH and ABTS free radicals scavenging activities in all microgreens. Significantly lower content of nitrate was obtained with LED 150 treatment. Finally, LED lamps have the potential to be used as the main light source for growing high nutritional quality microgreens in greenhouses.

Waralee Phansurin *et al.*, (2017) state that white LED light could be more effective at supporting petunia plant growth than RB LED light because of its greater ability to transmit through leaves and drive photosynthesis at the canopy level.

Weiguo Fu *et al.*, (2012) stated that (1) judged by the dynamics of anti-oxidative enzyme activity, there was no light stress to occur in the 100, 200 and 400 μmol/m2s treatments, a mild light stress occurred in the 600 μmol/m2s treatment, and a serious light stress occurred in the 800 $\mu mol/m^2s$ treatment; (2) increased light intensity gradually reduced the contents of soluble protein and nitrate in lettuce, whereas the content of soluble sugar remarkably increased. The biomass of a single plant of lettuce in the 600 $\mu mol/m^2s$ treatment was the highest and second highest in the 400 $\mu mol/m^2s$ treatment but was the lowest in the 100 $\mu mol/m^2s$ treatment. No significant difference in the biomass of single plant was observed between the 400 and 600 $\mu mol/m^2s$ treatments. Based on these results, the range of 400 $\mu mol/m^2s$ to 600 $\mu mol/m^2s$ is a recommendable light intensity for lettuce production.

Wong *et al.*, (2020) stated Photo morphogenesis refers to the growth and development of plants. Photoperiodism is the ability of plants to track time. Phototropism enables plants to grow towards or away from a light source.

Xiaoyan Zhang *et al.*, (2019) stated the effects of different LED light spectra on the growth, phenolic compounds profile, antioxidant capacity, and transcriptional changes in genes regulating phenolic biosynthesis in soybean microgreens were investigated. The results showed that light illumination decreased the seedling length and yield but increased phenolic compound content. Blue light and ultraviolet-A (UV-A) induced significant increases in total phenolic and total flavonoid content, as compared with the white light control. Sixty-six phenolic compounds were identified in the soybean samples, of which isoflavone, phenolic acid, and flavones were the main components. Ten phenolic compounds obtained from the orthogonal partial least-squares discriminant analysis (OPLS-DA) were reflecting the effect of light spectra. The antioxidant capacity was consistent with the phenolic metabolite levels, which showed higher levels under blue light and UV-A compared with the control. The highest transcript levels of phenolic biosynthesis-related genes were observed under blue light and UV-A. The transcript levels of *GmCHI*, *GmFLS*, and *GmIOMT* were also upregulated under far-red and red light. Taken together, our findings suggested that the application of LED light could pave a green and effective way to produce phenolic compound-enriched soybean microgreens with high nutritional quality, which could stimulate further investigations for improving plant nutritional value and should have a wide impact on maintaining human health.

YanqiZhan *et al*., (2021) stated that microgreens are attracting more consumers' attention due to their high nutritional value and unique sensory characteristics. This review focuses on the nutrition quality, sensory evaluation, pre- and post-harvest interventions, and health benefits of microgreens. Microgreens are rich in vitamins (e.g., vitamin C), minerals (e.g., copper and zinc), and phytochemicals, including carotenoids and phenolic compounds, which act as antioxidants in human body. Pre-harvest interventions, such as illumination, salinity stress, nutrient fortification, and natural substrates, influence the photosynthetic and metabolic activities of microgreens and were shown to improve their nutritional quality, while the effects varied among species. After harvesting, packaging method and storage temperature can influence the nutrient retention in microgreens. Both *in vitro* and *in vivo* studies have shown that microgreens have anti-inflammatory, anti-cancer, and anti-bacterial making it a new functional food beneficial to human health. The sensory attributes and overall acceptability and liking of microgreens are primarily influenced by their phytochemical content. Microgreens are only getting popular during the last decades and research on microgreens is still at its early stage.

Zeiger *et al*., (2002) stated that BL influences photosynthetic activity by inducing stomatal opening (Kasahara *et al*., 2002) affecting chloroplast movement within the cell (Hogewoning *et al*., 2010; *Wang et al*., 2016) in the short term while increasing stomata number and leaf thickness in the long term.

Zhonghua Bian *et al*., (2018) stated that the highest fresh and dry weight and leaf area were observed under red and blue LED light, with the blue light percentage at 23%. Compared with fluorescent lamps (FL) with photosynthetic photon flux density (PPFD) at 220 µmol m-2 s-1, the light-use efficiency increased by 55, 114 and 115% for mixed red and blue LEDs with PPFD at 100, 150 and 220 µmol m-2 s-1, respectively. The effect of light spectrum composition on lettuce nutrition quality was also studied. Continuous light with combined red, green and blue LEDs exhibited a remarkable decrease in nitrate. Moreover, continuous LED light for 24 h significantly increased phenolic compound content and free-radical scavenging capacity in lettuce leaf.

CHAPTER III

MATERIALS AND METHODS

This research work was conducted at the roof top (indoor structure) of Dr. M. Wazed Mia Research Centre, Sher-e-Bangla Agricultural University, Dhaka during the period from November 2020 to December 2020. Brief descriptions of materials and methods that are used in carrying out the experiment have been presented in this chapter.

3.1 Description of the experimental site

3.1.1 Experimental period

The experiment was conducted during the period from November to December 2020.

3.1.2 Experimental location

The experiment was conducted on FAB LAB at the Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. It is located on the 5th floor of Dr. M. Wazed Miya Research Centre at the Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

3.1.3 Indoor climatic Condition

In experiments, performed in controlled-environment growth chambers, day/night temperatures of 23±1°C, 16-h photoperiod and a relative air humidity of 60-64% were maintained.

3.2 Experimental details

3.2.1. Treatments

The experiment comprised of single factors:

Five different types of LEDs light:

1. L_1= White Light -100%

2. L_2= Red Light- 100%

3. L_3= Blue Light – 100%

4. L_4= Red & Blue Light -70:30

5. L_5= Red, Green &Blue Light- 70: 10: 20

Four different crops are used under this light concentration:

1. C_1= Mustard

2. C_2= Lettuce

3. C_3= Radish

4. C_4= Broccoli

There were five treatments, namely-

1. L_1= White Light -100%

2. L_2= Red Light- 100%

3. L_3= Blue Light – 100%

4. L_4= Red & Blue Light -70:30

5. L_5= Red, Green &Blue Light- 70: 10: 20

3.2.2 Preparation of different concentration of LEDs light

Microgreens were cultivated under custom-made lighting equipment containing five separate modules for parallel growth runs under individually controlled illumination conditions. Each module contained the main set of high-power LEDs with different PPFD. The main photosynthetic photon flux was provided by the set of white, red, blue, red and blue, and red, green and blue light with 150, 81, 224, 248 to 89 µmol.$m^{-2.}$ s^{-1}. Each module illuminated an area of 0.22 m^2 sufficient for simultaneous growth of plants in amounts large enough for the acquisition of statistically reliable data. PPFD was measured and regulated at the crop level using a photometer – radiometer. The light duration was 16 hours per day maintained by timer started at 6:00 am to 10:00 pm. All this LED light treatments were set up in several rakes vertically where each rake treated with any one of this treatment. Each treatment of the experiment was set up vertically in 5 racks and each rack contained four boxes.

3.3 Cultivation of crop

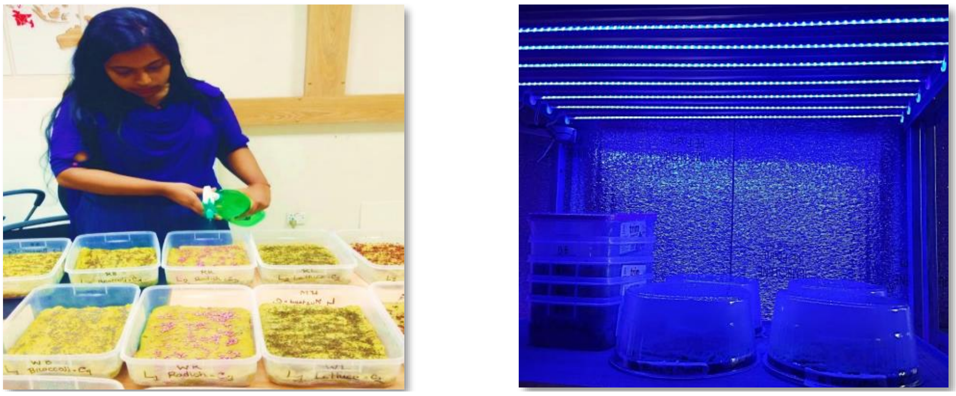
3.3.1. Collection of planting materials

Seeds of four types of microgreens mustard (*Brassica juncea*), Lettuce, Radish (*Raphanus raphanistrum* subsp. Sativas) and Broccoli *(Brassica oleracea var. capitata)* (Plate 1) were (*Lactuca scariola* var. *sativa*), collected from the local seed market to be used as plant materials. Seeds were sown in Rockwool (Basalt rock and Recycled Slag) in 2.5L plastic vessels (22×14 cm^2) for 9-10 days from germination to harvest. 10 g seeds of each crop were sown per vessel. Plants were watered daily (with a hydroponic solution) using a hand sprayer.

Plate 1. Seeds of selected crops

3.3.2 Seed germination

For germination 10g of seeds of each crop were taken into four different bowls full of water to select viable seed that laying on the bottom of bowl and then broadcast in the seed box for uniform distribution. To breakdown seed coat and to help germination all the selected seeds were taken into water for 4 hours. On the other side, germination media (rock wool) and the indoor room by controlling temperature and humidity were prepared. In each box water was sprayed daily @1spray/day for 4days (Plate 2. A). The box is covered with a plastic cover to create heat for germination and placed in indoor room (Plate 2. B).



A **B**

Plate 2. Preparation of seed germination: **A.** After seed sowing spraying the hydroponic solution. **B.** Placing seed box in indoor room

3.3.3 Media Preparation

Rock wool sheet cutting with 24 × 22 cm^2 as germination media cutting was used in order to the area of germination. By placing the sheets into the clear box, germination media was prepared. Then the sheets soaked with hydroponic solution overnight then the sheets were placed in box. After that the growing media (Rock wool) was prepared. Then 10g seeds per rock wool sheet was sowed. Then these sheets were covered with some heavy materials (one box upon another) to warm the area which easily doing germination. All of these boxes were taken under ambient room condition. Some hydroponic solution was also offered to keep the media moisturized. Moisture condition was checked by pressing the media by finger.

3.3.4. Seedling emergence

The apparent, fifty and ninety percent seedling emergence took place within 3 days after sowing for all selected microgreens crops.

3.4 Design and layout of the experiment

The experiment was laid out in a randomized complete block design (RCBD) having Five story Iron Rack (20 treatment combinations with three replications). The experiment was carried out on the five layers of the iron rack. In each layer four boxes were placed. The box-to-box distance was 8cm. No space was provided within box as it didn't not require any space for intercultural operations. About 10g of seeds, sown in peat moss in Rockwool tray in a controlled conditions greenhouse (3 trays per each variety for 3 replicates), cultivated under relative humidity (RH) of 60-70% and temperature ranges from 22-24°c. Each day, 100 ml of hydroponic solutions was added to each tray to further stimulate seedling growth. Once cotyledons will fully be reflexed 5 d after sowing, 300 ml of 25% hydroponic solutions was added to each tray daily until harvest. A picture of the Light set up and a part of the experimental set up stated below:

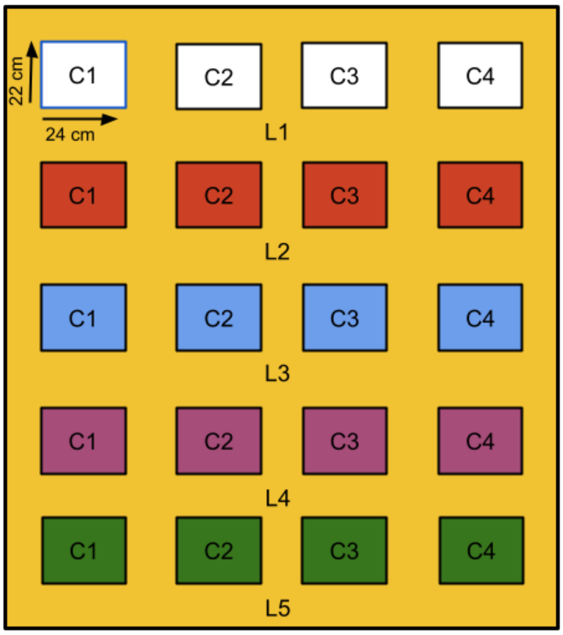
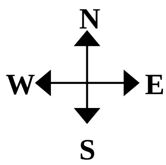


Figure 1. Layout of the experimental plot

Legend



Here,
C1= Mustard
C2= Lettuce
C3= Broccoli
C4= Radish

Treatment: Five different concentrations of LEDs light:
L_1= White Light (100)
L_2= Red Light (100)
L_3= Blue Light (100)
L_4= Red & Blue Light (70:30)
L_5= Red, Green &Blue Light (70:10:20)

Here,

Number of Layers: 5

Layers spacing: 1m

Number of Box: 20

Box size: 24×22cm^2

3.5 Irrigation

Irrigation was given on everyday using a spray bottle. Approximately 125ml hydroponic solution (KNO_3-25.28ml/L, $Ca(NO_3)_2$-70.85ml/L, $MgSO_4$-36.97ml/L, KH_2PO_4-13.61ml/L, NH_4CL-53.50g/L, H_3BO_3 -3.092ml/L, MnCL-1.98ml/L$ZnSO_4$ -0.567ml/L) provided each of the growing boxes. So that, the rock wool was not got dry.

A

B

Plate 3. Steps after germination: **A.** Emergence of seedling **B.** Irrigating with hydroponic solutions

3.6 Harvesting

Microgreens are harvested at 12days after sowing. All plants are harvested from every box of each treatment combinations (Plate 4) and were used for data observation and recording regarding yield performance.

3.7 Data collection

Fifteen plants were randomly selected from each of the treatment combination. Data on different growth parameter, such as- Hypocotyl length, Chlorophyll content were collected at 3, 6, 9 and 12 days after sowing (DAS). Yield parameters were recorded at harvest which included fresh weight and yield per device. Total yield was calculated from yield per device. Fresh and dry weights of individual plant were recorded after harvest. Data were collected on the following parameters:

- Hypocotyl length(cm)
- SPAD value(nm)
- Fresh weight (gm)
- Dry weight (gm)
- Nitrate Content (%)
- Potassium Content (%)
- Brix (%)
- Antioxidant (%)

3.8 Procedure of recording data

3.8.1 Hypocotyl length

The hypocotyl length measured below seed leaf. It is the stem of a germinating seedling, found below the cotyledons (seed leaves) and above the radicle (root). It was measured with a meter scale (Plate 6) from ten randomly selected plants at 3days interval beginning at 3days after sowing (DAS) and continued till harvest. The units were expressed in cm.

3.8.2 SPAD value

SPAD value was determined at harvesting time using SPAD meter (Plate 6 and 9). Data is collected from ten plants from a box of each treatment.

3.8.3 Yield/Box

Yield of microgreens was recorded at final harvest within a box (Plate 5) and was expressed in gram

3.8.4 Fresh Weight of all plants per box

Weighted all of the crops of each box and recorded (Plate 6). It was measured in grams (g) with an electric balance.

3.8.5 Dry matter content per box

At first, plants were collected, cut into pieces and sun dried for 72 hours followed by drying in an oven at 70°C for 72 hours. The weight of the oven dried sample was taken (Plate 6 and 9) and express in gram.

3.8.6 Nitrogen content (%)

Nitrogen content was measured with the salicylic sulphuric acid method. 10 mg of oven-dried samples (80°C for 48 h) were suspended in 10 mL of distilled water and left in agitation for 2 h. After that, 20 L of sample were added to 80L of 5% salicylic acid in sulphuric acid and to 3 mL of NaOH 1.5 N. Samples were cooled at room temperature and the spectrophotometer readings were performed at 410 nm. Nitrogen content was calculated referring to a KNO_3 standard calibration curve (Plate 7). Data were expressed on a fresh weight (FW) basis considering the fresh weight/dry weight ratio.

3.8.7 Brix% (°Brix)

Brix% was measured by a refractometer (ERMA, Tokyo, Japan) at room temperature. At very first stage microgreens was collected and taken in a mortar and blended with the help of pistol for extracting juice. Then the extract put on the refractometer and recorded the %brix (Plate 6).

3.8.8 Potassium content (%)

For assessing the potassium content, oven-dried samples (80°C for 48 h) were ground and digested with nitric acid, and elements were measured using inductively coupled plasma mass spectroscopy (ICP-MS) (Plate 7 and 9). Data were expressed on an FW basis considering the fresh weight/dry weight ratio.

3.8.9 Antioxidant (%)

The antioxidant activity of the ethanolic extracts was determined on the basis of their scavenging activity of the stable 1,1–diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radicle containing an odd electron in its structure and usually utilized for detection of the radicle scavenging activity in chemical analysis. 1ml of each solution of different concentrations (1-500 µg/ml) of the extracts was added to 3ml of 0.004% ethanolic DPPH free radicle solution. After 30 minutes the absorbance of the preparations was taken at 517 nm by a UV spectrophotometer which was compared with the corresponding absorbance of standard ascorbic acid concentrations (1 -500 µg/ml) (Plate 8 and 9). The method described by (Hatano *et al*., 2006) was used to measure the absorbance with some modifications. Then the % inhibition was calculated by the following equation:

$$\%\text{radical} = \frac{(\text{Absorbance of blank} - \text{Absorbance of scavenging activity sample})}{\text{Absorbance of blank}} \times 100$$

From calibration curves, obtained from different concentrations of the extracts, the IC_{50} (Inhibitory concentration 50%) was determined. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicles.

3.9 Statistical analysis

The data obtained for different characters were statistically analysed to find out the significance of different light intensity for different microgreens variety and growth and yield of microgreens. The mean values of all the recorded characters were evaluated and analysis of variance was performed. The significance of the difference among the treatment combinations of means was estimated by Least Significant Difference (LSD) value at 5% level of probability.

3.10 Economic Analysis

The economic analysis was done by calculation of production cost and price of the produce in order to find out the most economic combination of different light intensity for selected microgreens crops in indoor room. All input cost and interests on running capital was included in computing the cost of production. The interests were calculated @ 13% in simple rate. The market price of microgreens crops was considered for estimating the gross and net return. Economic analyses were done according to the procedure of Alam *et al*. (1989). The benefit cost ratio (BCR) was calculated as follows:

$$\text{Benefit Cost Ratio (BCR)} = \frac{\text{Gross return (tk)}}{\text{Total cost of production (tk)}}$$

A

B

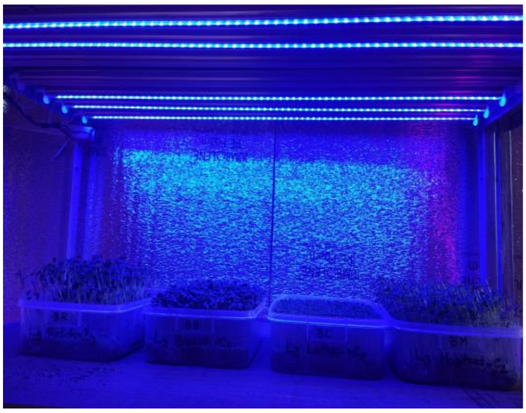
C

D

E

Plate 4. Harvesting stage of selected microgreens crops under different lights combinations. **A.** L_1= White light (100%) **B.** L_2= Red light (100%) **C.** L_3= Blue light (100%) **D.** L_4=Red: Blue light (70:30) **E.** L_5= Red: Green: Blue light (70:10:20)

A **B**



C **D**

E

Plate 5. Yield of microgreens under different LEDs light combination: **A.** L_1= White light (100%) **B.** L_2= Red light (100%) **C.** L_3= Blue light (100%) **D.** L_4=Red: Blue light (70:30) **E.** L_5= Red: Green: Blue light (70:10:20)

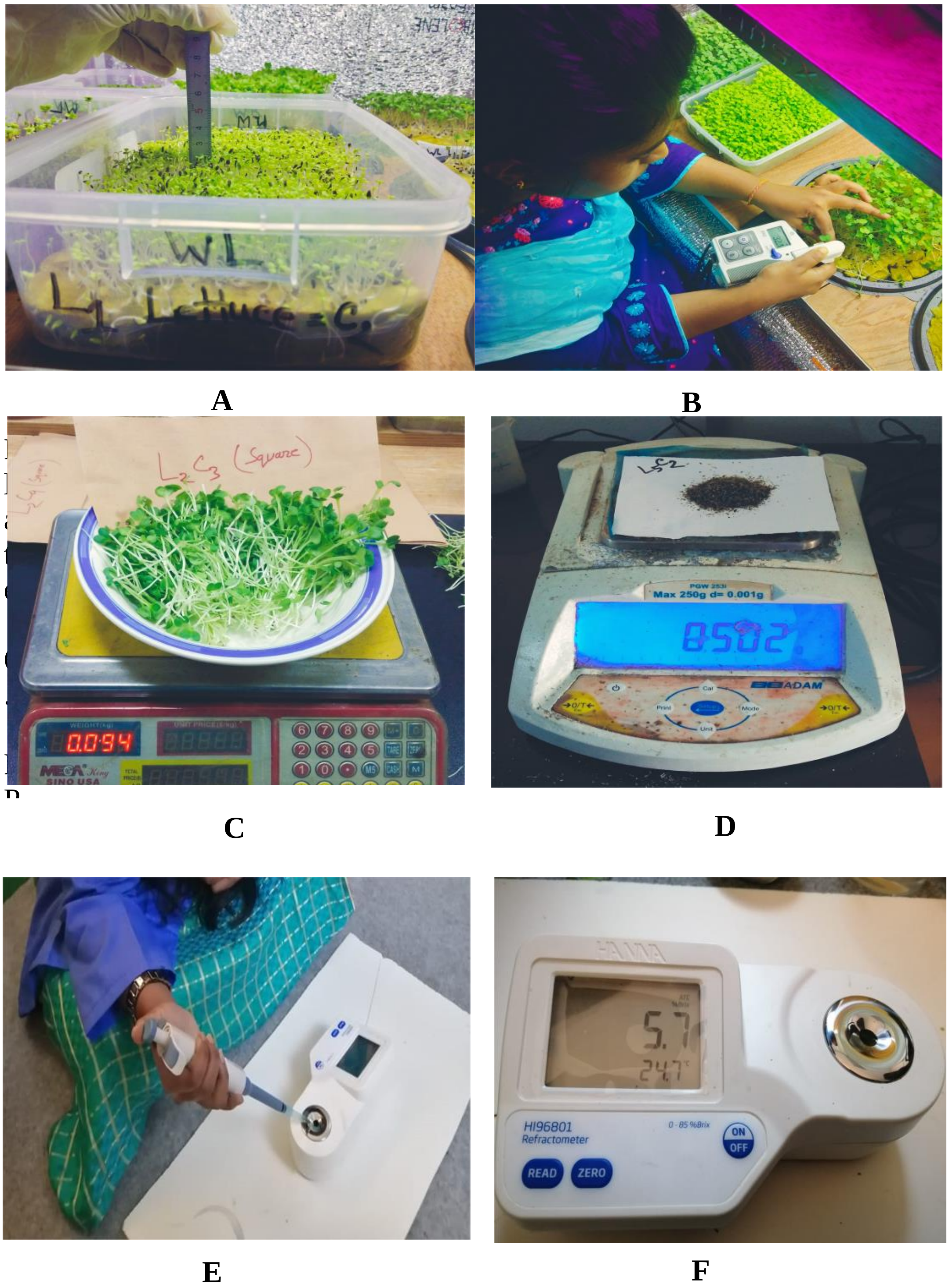


Plate 6. Pictorial presentation of experiment: **A.** Recording hypocotyl length **B.** Recording SPAD value **C.** Weighting fresh weight **D.** Weighting dry weight **E.** Plant extract putting on refractometer **F.** Recording °Brix value

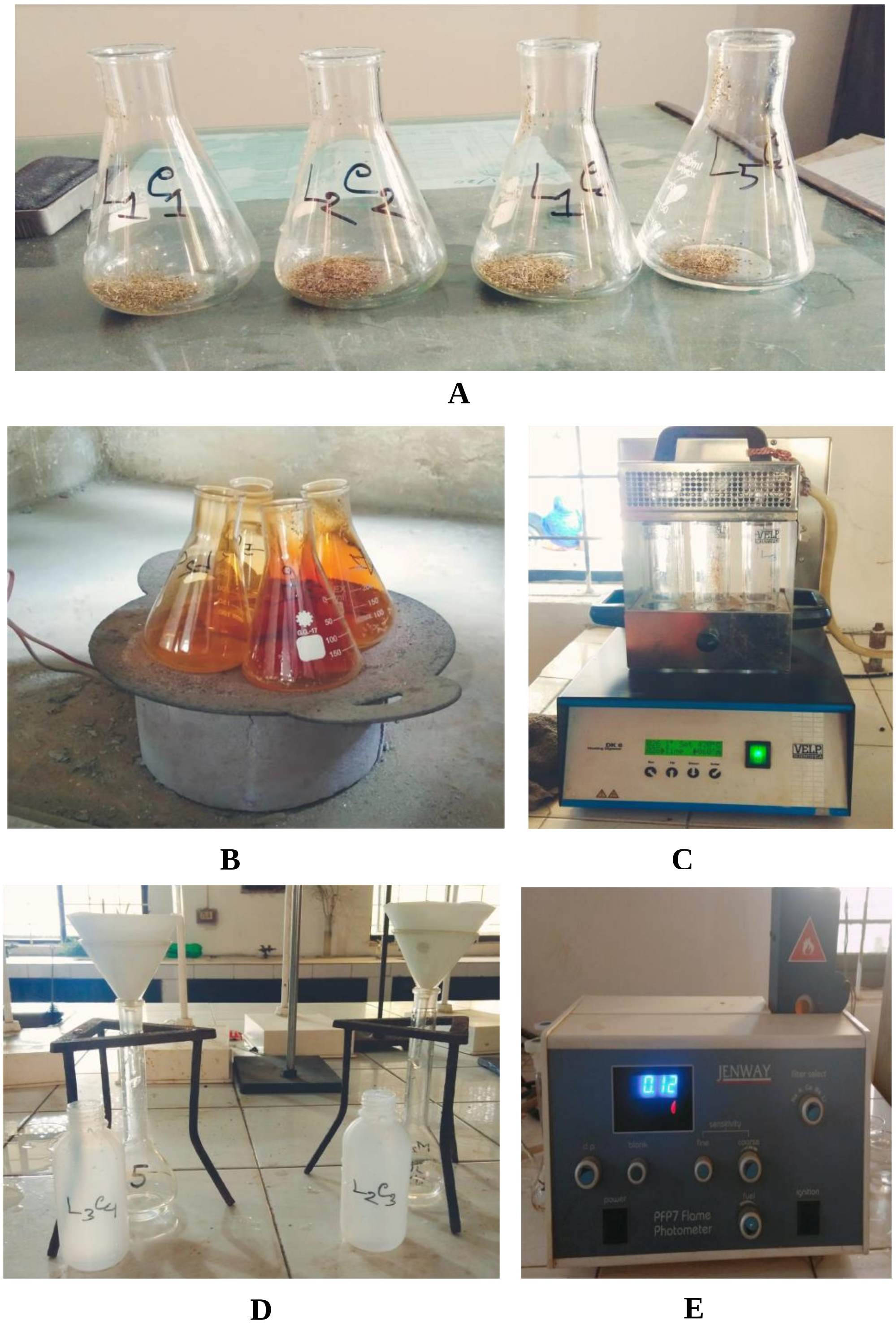


Plate 7. Procedure of collecting data of nitrogen and potassium content: **A.** Took dry sample **B.** Heating the solution **C.** Digestion the solution **D.** Filtering the solution **E.** Took Flame photometer reading of potassium content

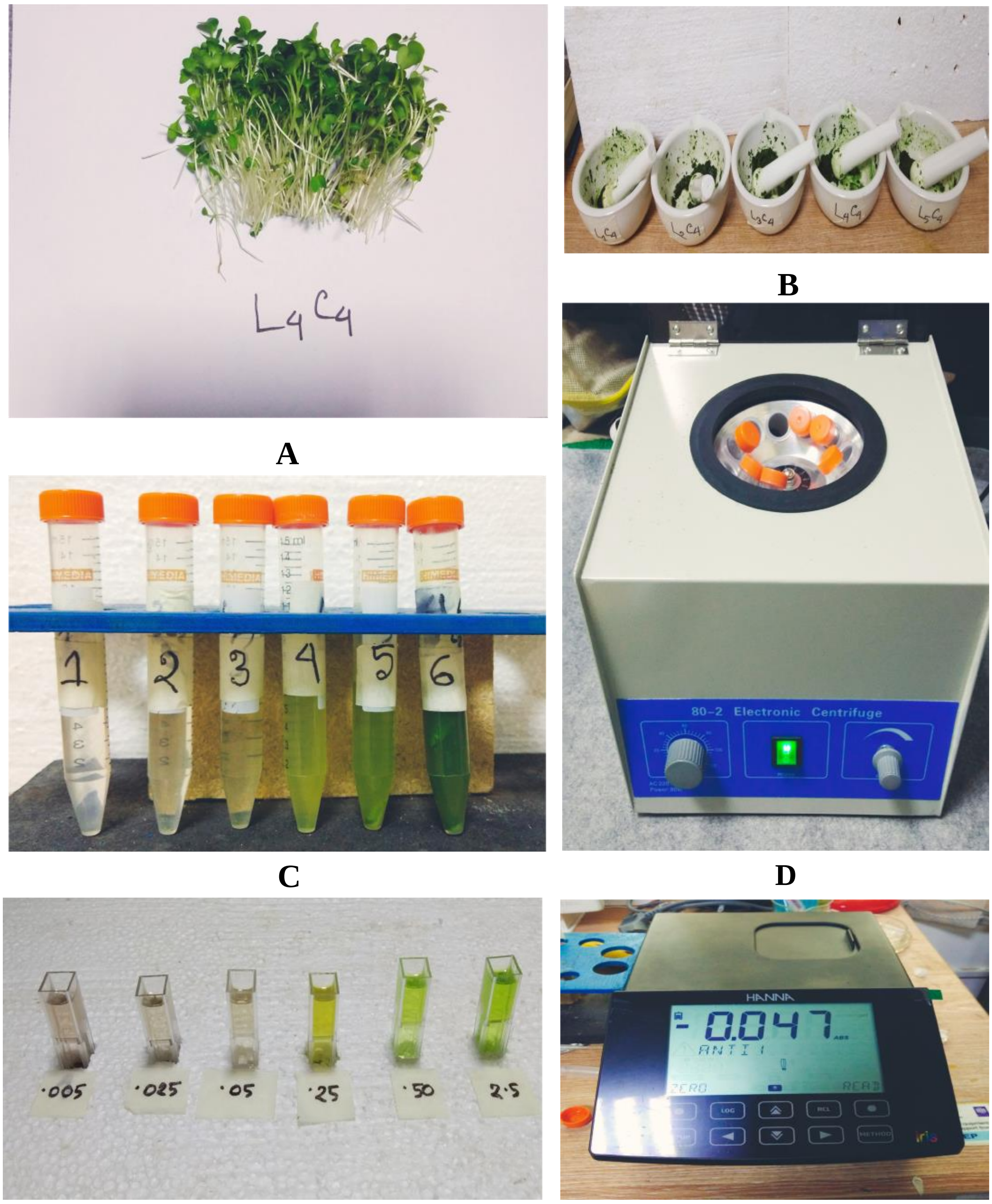


Plate 8. Procedure of collecting data of antioxidant: **A.** Took fresh sample **B.** Pasting the sample **C.** Made solution for centrifugal pump **D.** Solution placed in centrifugal pump **E.** Solution of testing antioxidant **F.** Spectrophotometer reading of antioxidant

CHAPTER IV

RESULTS AND DISUSSIONS

The experiment was conducted to evaluate the development of microgreens produces and evaluations of growth and nutritional profile under different LEDs light combination. Data were collected on various growth and yield indicator and data was statistically analyzed with Statistix10.0 software. Appendices VII-XIV contains the analysis of variance (ANOVA) of the data on different growth and yield parameters. Figures, graphs and tables were used to discuss the findings of the study as well as their most probable interpretation in this chapter under the following headings:

4.1 Growth parameters

4.1.1 Hypocotyl Length

Significant variation was found for hypocotyl length of mustard, lettuce, broccoli and radish at different growth stages as influenced by different LED-light spectral ratios (Figure 1, and Appendix III). Hypocotyl length of four different crops (Radish, Mustard, Broccoli and Lettuce) as factor B at 3, 6, 9 and 12 (DAS) and had statistically significant variation due to different combination of LEDs light treatment. Results exhibited that the highest hypocotyl length of mustard, lettuce, broccoli and radish at 3 DAS respectively (4.57, 2.97, 6.47 and 3.57cm) was recorded from the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue -70: 30) which was significantly different from other treatments followed by L_5C_1, L_5C_2, L_5C_3, and L_5C_4 (Red: Green: Blue -70: 10: 20), and L_3C_1, L_3C_2, L_3C_3 and L_3C_4 (Blue - 100%) whereas the lowest hypocotyl length (3.13, 2.1, 4.63 and 2.03 cm) was found from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White - Full) which was statistically identical with L_2C_1, L_2C_2, L_2C_3 and L_2C_4 (Red - 100%). Similar trend was found for hypocotyl length at 6, 9, 12 DAS, the highest hypocotyl length of mustard, lettuce, broccoli and radish at 6 DAS respectively (7.83, 5.23, 11 and 7.47cm) was recorded from the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue - 70: 30) which was significantly different from other treatments followed by L_5C_1, L_5C_2, L_5C_3, and L_5C_4 (Red: Green: Blue - 70: 10: 20), and L_3C_1, L_3C_2, L_3C_3 and L_3C_4 (Blue - 100%) whereas the lowest hypocotyl length (5.57 cm, 3.7 cm, 7.87 cm and 5.07 cm) was found from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White - Full) which was statistically identical with L_2C_1, L_2C_2, L_2C_3 and L_2C_4 (Red -100%). At 9 DAS, L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue -70: 30) gave the highest hypocotyl length (9.63cm, 6.9cm, 13.93cm and 9.83cm) which was statistically identical with L_5C_1, L_5C_2, L_5C_3, and L_5C_4 (Red: Green: Blue -70: 10: 20), and L_3C_1, L_3C_2, L_3C_3 and L_3C_4 (Red and Blue -70: 30) whereas the lowest hypocotyl length (7, 4.83, 10.2 and 6.73 cm) was found in the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White - Full) which was statistically identical with L_2C_1, L_2C_2, L_2C_3 and L_2C_4 (Red – 100%). Again, at 12 DAS the highest hypocotyl length (10.53, 8.47, 15.23 and 11.43 cm) from the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue - 70:30) which was statistically identical with L_5C_1, L_5C_2, L_5C_3, and L_5C_4 (Red: Green: Blue - 70: 10: 20), and L_3C_1, L_3C_2, L_3C_3 and L_3C_4 (Red and Blue -70:30) whereas the lowest hypocotyl length (7.67, 5.63, 11.2 and 7.73 cm) was found in the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White - Full) which was statistically identical with L_2C_1, L_2C_2, L_2C_3 and L_2C_4 (Red – 100%). As a result, briefly it was found that at 3, 6, 9 and 12 DAS, the highest hypocotyl length for mustard (4.57, 7.83, 9.63, and 10.53 cm, respectively), for lettuce (2.97, 5.23, 6.9 and 8.47 cm), for broccoli (6.47, 11, 13.93 and 15.23 cm) and for radish (3.57, 7.47, 9.83 and 11.43 cm) were found for the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue -70:30) followed by L_5C_1, L_5C_2, L_5C_3, and L_5C_4 (Red: Green: Blue -70: 10: 20), whereas the lowest hypocotyl length for mustard (3.13, 5.57, 7, and 7.67 cm, respectively), for lettuce (1.83, 3.7, 4.83 and 5.63 cm), for broccoli (4.63, 7.87, 10.2 and 11.2 cm) and for radish (2.03, 5.07, 6.73 and 7.73 cm) were found from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White - Full) which was statistically identical with L_2C_1, L_2C_2, L_2C_3 and L_2C_4 (Red – 100%).

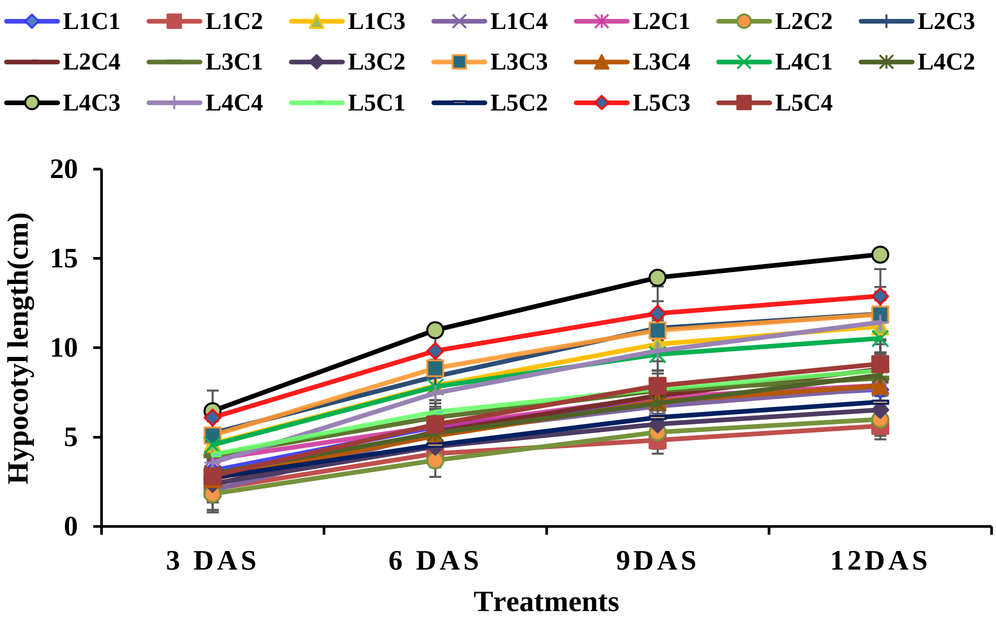


Figure 2. Graphical representation of hypocotyl length of selected crops at different growth stages (3, 6, 9 and 12 DAS) influenced by different LED-light spectral ratios.

Here, 1. L_1= White Light -100%, 2. L_2= Red Light- 100%, 3. L_3= Blue Light – 100%, 4. L_4= Red & Blue Light -70:30, 5. L_5= Red, Green &Blue Light- 70: 10: 20, A. C_1= Mustard, B. C_2= Lettuce, C. C_3= Radish and D. C_4= Broccoli

The result obtained from the present study was similar with the findings of (Ausra Brazaityte *et al*., 2018) and they found that light supplementation can increase crop yield in greenhouses by promoting photosynthesis and plant growth and also obtained higher hypocotyl length with higher percentage of blue LED light in microgreens. Similar result was also observed by (Joshua K. Craver *et al*., 2017).

4.2 Yield Contributing Parameter

4.2.1 Fresh weight (g)/box

Significant variation was recorded on fresh weight of mustard, lettuce, broccoli and radish as affected by different LED-light spectral ratios (Table 1 and Appendix IV). Results revealed that the highest fresh weight of mustard (100g), lettuce (100g), broccoli (100g) and radish (100g) were achieved from the treatment L_4C_1 , L_4C_2, L_4C_3, and L_4C_4 (Red: Blue - 70:30) which were significantly different from other treatments followed by L_5C_1, L_5C_2, L_5C_3 and L_5C_4 (Red : Green : Blue -70: 10: 20), whereas the lowest fresh weight plant for mustard(72g), lettuce (92.33g), broccoli (93.3g) and radish (91.33g) were recorded from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White - Full) which were statistically identical with L_2C_1, L_2C_2, L_2C_3 and L_2C_4 (Red – 100%). The result of the present study was in agreement with the findings of Brazaityte *et al*. (2018) and reported that various mineral elements content was mostly caused by higher percentage of blue light respectively. Brazaityte *et al*. (2018) also found similar result with the present study and found that Red and blue LEDs increased fresh biomass of Brassicaceae microgreens. Bian *et al*. (2018) also found highest fresh and dry weight and leaf area under blue LED light, with the red and blue light (70:30) compared with fluorescent lamps (FL) which was similar with the result of the present study.

4.2.2 Total yield (kg)

Statistically significant difference among the treatment was found on total yield of selected microgreens in the experiment area per treatment as influenced by different LED-light spectral ratios (Appendix IV). Results exhibited that the highest total yield (0.115 kg, 0.110kg, 0.135kg and 0.125kg) were found from the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue -70:30) which was significantly similar with the treatment L_5C_1, L_5C_2, L_5C_3, and L_5C_4 (Red: Green: Blue -70: 10: 20). Reversely, the lowest total yield (0.078 kg, 0.090kg, 0.098kg and 0.1kg) were recorded from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White - Full) which was significantly different from other treatments. Specht *et al*. (2014) reported that vertical farming facilitates the production of high value crops with higher yield than obtained from conventional farming. In such a case, LED lights are more effective with high energy use efficiency and durability than traditional light sources like fluorescent lamps (Specht *et al*., 2014). A blue and red background (B16R84) with 24% GL from green fluorescent lamp

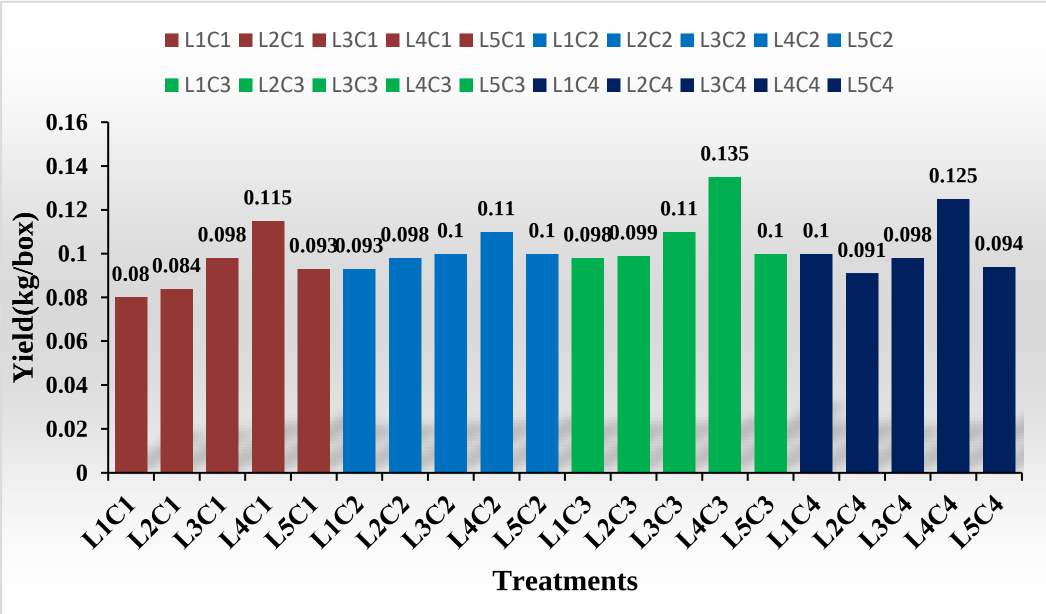


Figure 3. Graphical view of Yield(kg/box) of selected crops influenced by different LED-light spectral ratios.

Here, 1. L_1= White Light -100%, 2. L_2= Red Light- 100%, 3. L_3= Blue Light – 100%, 4. L_4= Red & Blue Light -70:30, 5. L_5= Red, Green &Blue Light- 70: 10: 20, A. C_1= Mustard, B. C_2= Lettuce, C. C_3= Radish and D. C_4= Broccoli

(B15G24R61) has been shown to increase lettuce yield (Kim *et al.*, 2004), whereas in another study, an increase of up to 30% green LED light does not influence the dry mass in the same cultivar (Snowden *et al.*, 2016). Piovene *et al.* (2015) found that the plants expressed increased biomass and fruit yield of basil and strawberry when treated with LED with highest energy use efficiency than traditional fluorescent lamps and spectral red: blue ratio of 0.7 was essential for proper plant growth and improved nutraceutical properties. It was reported that by Wong (2020) that lighting quality and quantity can be manipulated to improve yield and phytonutrient contents of leafy greens.

4.2.3 Dry weight (g)

Significant variation was recorded on Dry weight of mustard, lettuce, broccoli and radish as affected by different LED-light spectral ratios (Table 1 and Appendix IV). Results revealed that the highest dry weight of mustard (4.33g), lettuce (3.5g), broccoli (4.6g) and radish (5.63g) were achieved from the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue -70:30) which were significantly different from other treatments followed by L_5C_1, L_5C_2, L_5C_3, and L_5C_4 (Red : Green: Blue - 70: 10: 20), whereas the lowest dry weight of mustard (1.367g), lettuce (1.233g), broccoli (2.63g) and radish (2.6g) were recorded from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White - Full) which were statistically identical with L_2C_1, L_2C_2, L_2C_3 and L_2C_4 (Red – 100%). The result of the present study was similar with the findings of Brazaityte *et al*. (2018) and reported that various mineral elements content was mostly caused by higher percentage of red and blue light. Bian *et al*. (2018) also found highest fresh and dry weight and leaf area under red and blue LED light, with the ratio of 70:30 compared with white light which was similar with the result of the present study.

Table 1. Fresh and dry weight of selected crops at harvest influenced by different LED-light spectral ratios

Treatment Combination	**Fresh weight (gm)**	**Dry weight (gm)**
L1C1	72c	1.37g
L2C1	76.67bc	1.53g
L3C1	88.33ab	2.07f
L4C1	100a	4.33b
L5C1	93.33a	2.43ef
L1C2	92.33a	1.33g
L2C2	95a	1.53g
L3C2	96.67a	1.23g
L4C2	100a	3.5c
L5C2	99.33a	3.03d
L1C3	93.33a	3.6c
L2C3	98.67a	2.63e
L3C3	99.33a	4.33b
L4C3	100a	4.6b
L5C3	100a	4.23b
L1C4	91ab	2.6e
L2C4	91.33ab	2.63e
L3C4	97.67c	3.4cd
L4C4	100a	5.63a
L5C4	98.33a	3.77c
SE (±)	7.44	0.19
$LSD_{(0.05)}$	15.06	0.37
CV (%)	9.81	7.57

In a column means having similar letter(s) arc statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

Here, 1. L_1= White Light -100%, 2. L_2= Red Light- 100%, 3. L_3= Blue Light – 100%, 4. L_4= Red & Blue Light -70:30, 5. L_5= Red, Green &Blue Light- 70: 10: 20, A. C_1= Mustard, B. C_2= Lettuce, C. C_3= Radish and D. C_4= Broccoli

4.3 Quality Parameter

4.3.1 SPAD value

SPAD value of mustard, lettuce, broccoli and radish showed statistically significant variation as affected by different LED-light spectral ratios (Figure 2). Results revealed that the highest SPAD value of mustard (38.2nm), lettuce (16.9nm), broccoli (55.3nm) and radish (49.9nm) were achieved from the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue -70:30) which was significantly different from other treatments followed by L_5C_1, L_5C_2, L_5C_3, and L_5C_4 (Red : Green: Blue -70: 10: 20), and L_3C_1, L_3C_2, L_3C_3, and L_3C_4 (Blue-100%), whereas the lowest SPAD value for mustard(29.5nm), lettuce (12nm), broccoli (41.4nm) and radish (40.6nm) were recorded from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White Light - Full) which was statistically identical with L_2C_1, L_2C_2, L_2C_3 and L_2C_4 (Red – 100%). This result indicated that the red and blue light is responsible for higher chlorophyll content. BL influences photosynthetic activity by inducing stomata opening (Zeiger *et al*., 2002) and affecting chloroplast movement within the cell (Kasahara *et al*., 2002) in the short term while increasing stomata number and leaf thickness in the long term (Hogewoning *et al*., 2010; Wang *et al*., 2016). BL is also known to increase the chlorophyll content (Hogewoning *et al*., 2010; Johkan *et al*., 2010; Matsuda *et al*., 2007). A greater fraction of BL is associated with the development of ―sun-type‖ leaf characterized by a high leaf thickness and photosynthetic capacity (Hogewoning *et al*., 2010; Matsuda *et al*., 2007).

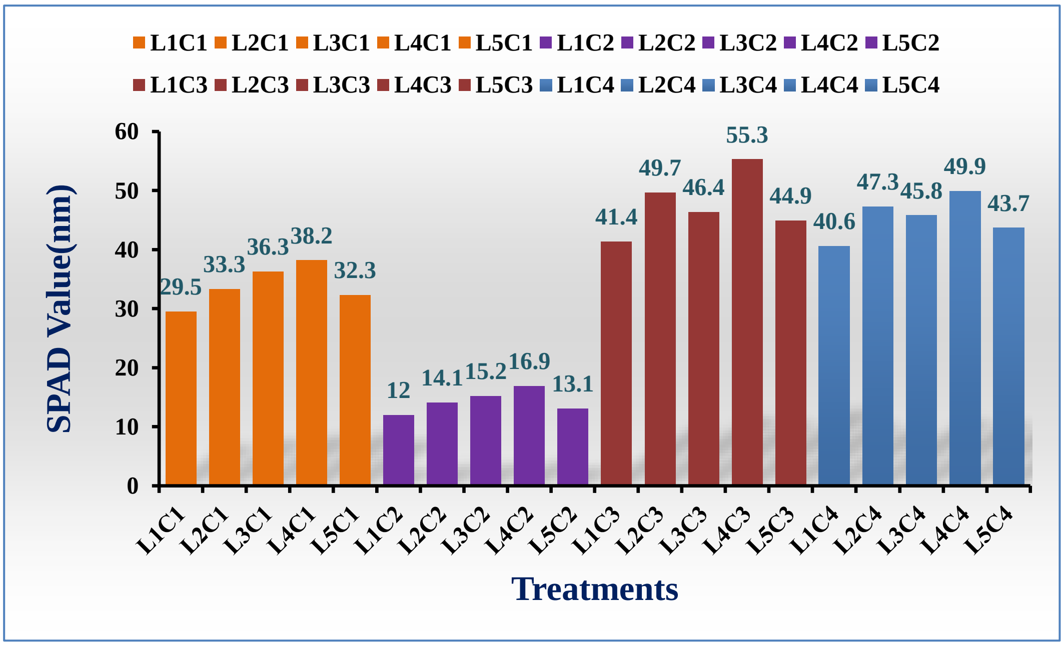


Figure 4: Graphical view of SPAD value (nm) in different treatments for the selected crops.

Here, 1. L_1= White Light -100%, 2. L_2= Red Light- 100%, 3. L_3= Blue Light – 100%, 4. L_4= Red & Blue Light -70:30, 5. L_5= Red, Green &Blue Light- 70: 10: 20, A. C_1= Mustard, B. C_2= Lettuce, C. C_3= Radish and D. C_4= Broccoli

4.3.2 Nitrogen Content (%)

Different LED-light spectral ratios showed significant influence on nitrate content of mustard, lettuce, radish and broccoli (Table 2 and Appendix V). The highest nitrogen content of mustard(38.23%), lettuce(16.93%), radish (55.27%) and broccoli (93.93%) were recorded from the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue -70: 30) which was significantly different from other treatments followed by L_5C_1, L_5C_2, L_5C_3, and L_5C_4 (Red : Green: Blue -70: 10: 20), and L_3C_1, L_3C_2 , L_3C_3 , and L_3C_4 (Blue-100%) whereas the lowest nitrogen content for mustard(29.5%), lettuce (12%), radish (41.43%) and broccoli(40.6%) were recorded from the control treatment L_1C_1 , L_1C_2 L_1C_3 and L_1C_4 (White Light - Full) which was statistically identical with L_2C_1, L_2C_2, L_2C_3 and L_2C_4 (Red – 100%). The result obtained from the present study on nitrogen content was similar with the findings of (YanqiZhan *et al*., 2021).

4.3.3 Potassium Content (%)

Different LED-light spectral ratios showed significant influence on potassium content of mustard, lettuce, radish and broccoli (Table 2 and Appendix V). The highest potassium content of mustard(0.19%), lettuce(0.19%), radish(0.22%) and broccoli (0.16%) were recorded from the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue -70:30) which was significantly different from other treatments followed by L_5C_1, L_5C_2, L_5C_3, and L_5C_4 (Red : Green: Blue -70: 10: 20), and L_3C_1, L_3C_2 , L_3C_3 , and L_3C_4 (Blue-100%) whereas the lowest potassium content for mustard(0.09%), lettuce (0.11%), radish (0.14%) and broccoli(0.1%) were recorded from the control treatment L_1C_1 , L_1C_2 L_1C_3 and L_1C_4 (White Light - Full) which was statistically identical with L_2C_1, L_2C_2, L_2C_3 and L_2C_4 (Red – 100%). The result obtained from the present study on nitrogen content was similar with the findings of (YanqiZhan *et al*., 2021).

4.3.4 Antioxidant Capacity (%)

Significant influence on antioxidant capacity of mustard, lettuce, radish and broccoli were statistically showed (Table 2 and Appendix V) under different LED-light spectral ratios. The highest antioxidant capacity of mustard (0.22 %), lettuce(0.23%), radish(0.19%) and broccoli (0.18%) were recorded from the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue -70:30) which was significantly different from other treatments followed by L_5C_1, L_5C_2, L_5C_3, and L_5C_4 (Red : Green: Blue -70: 10: 20), and L_3C_1, L_3C_2 , L_3C_3 , and L_3C_4 (Blue-100%) whereas the lowest antioxidant capacity for mustard (0.14%), lettuce (0.14%), radish (0.11%) and broccoli(0.09%) were recorded from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White Light - Full) which was statistically identical with L_2C_1, L_2C_2, L_2C_3 and L_2C_4 (Red – 100%). Microgreens has high level of nutrition components. It contains, vitamins, minerals and antioxidants compared to matured greens (Barbara B. *et al*., 2014). The result obtained from the present study on nitrogen content was similar with the findings of (YanqiZhan *et al*., 2021). (Masahumi *et al*., 2010) stated that Polyphenol contents and the total antioxidant status (TAS) were greater in lettuce seedlings treated with red and blue-containing LED lights than in those treated with FL. (Xiaoyan Zhang *et al*., 2019) also found similar result with the present study and found that blue LEDs light induced significant increases in antioxidant capacity of microgreens. (T G Shibaeva *et al*.,2022) also found similar result with the present study that was continuous LEDs lighting allow producers to increase antioxidant capacity of Brassicaceae microgreens.

4.3.5 Brix content (°Brix)

Brix content of selected microgreens varied significantly due to different LED-light spectral ratios (Table 2 and Appendix V). The maximum °Brix was found in mustard (4.6%), lettuce (4.4%), radish (6.17%) and broccoli (3.83%) were recorded from the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue - 70: 30) which was significantly different from other treatments followed by L_5C_1, L_5C_2, L_5C_3, and L_5C_4 (Red : Green: Blue -70: 10: 20), and L_3C_1, L_3C_2, L_3C_3, and L_3C_4 (Blue-100%) whereas the lowest °Brix for mustard (2.97%), lettuce (2.67%), radish (3.1%) and broccoli (3.27%) were recorded from the control treatment L_1C_1 , L_1C_2 L_1C_3 and L_1C_4 (White Light - Full) which was statistically identical with L_2C_1, L_2C_2, L_2C_3 and L_2C_4 (Red – 100%). The result obtained from the present study on °Brix was the highest in microgreens grown under BL and RL was similar with the findings of (Mickens *et al*., 2019).

Table 2. Quality parameters of selected crops at harvest influenced by different LED- light spectral ratios

Treatment Combination	Quality Parameters			
	Nitrate (%)	Potassium (%)	Antioxidant(%)	Brix (%)
L1C1	29.5k	0.09h	0.14d	2.97k
L2C1	32.33jk	0.14d	0.18b	4.07d
L3C1	33.33ijk	0.13b	0.18b	3.63fg
L4C1	38.23ghi	0.19de	0.22a	4.6b
L5C1	36.13hij	0.12ef	0.15cd	3.4hi
L1C2	12l	12l	0.14d	2.67l
L2C2	13.1l	0.14d	0.19b	3.47gh
L3C2	14.07l	0.14b	0.18b	3.2ij
L4C2	16.93l	0.19d	0.23a	4.4bc
L5C2	15.2l	0.13de	0.15cd	3.17jk
L1C3	41.43efg	0.14d	0.11ef	3.1jk
L2C3	44.9cdef	0.17c	0.15cd	4.33c
L3C3	46.4a	0.17a	0.19b	3.97de
L4C3	55.27bcde	0.22c	0.16c	6.17a
L5C3	49.7bc	0.14d	0.11ef	3.13jk
L1C4	40.6fgh	0.1gh	0.09g	3.27hij
L2C4	43.73def	0.14d	0.14d	3.63fg
L3C4	45.8b	0.12c	0.12ef	3.63fg
L4C4	49.93bcde	0.16ef	0.18b	3.83ef
L5C4	47.33bcd	0.12ef	0.1fg	3.4hi
SE (±)	2.45	7.96	8.83	0.11
$LSD_{(0.05)}$	4.97	0.02	0.018	0.23
CV (%)	0.56	6.82	6.95	3.7

In a column means having similar letter(s) arc statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability

Here, 1. L_1= White Light -100%, 2. L_2= Red Light- 100%, 3. L_3= Blue Light – 100%, 4. L_4= Red & Blue Light -70:30, 5. L_5= Red, Green &Blue Light- 70: 10: 20, A. C_1= Mustard, B. C_2= Lettuce, C. C_3= Radish and D. C_4= Broccoli

4.4 Economic analysis

Input costs included making and installation costs of devices price of different components of growing media (Rock wool), seed, LEDs light strip, hydroponic solution and man power required for all the operations from seed sowing to harvesting of microgreens crops were recorded as per box and converted for cost of production on (cost/ 0.029m^2) (Appendix). Price of microgreens crops was determined as per market rate basis of super shops of Dhaka city. The economic analysis is presented under the following headings-

4.4.1 Gross return

The gross return calculated from different LEDs light combinations and selected microgreens crops in (Table 3.) The highest (BDT 8337000, 7938000, 9786000 and 9051000/ ha) and second highest (BDT 7959000, 7392000, 8337000 and 7959000/ ha) gross return were obtained from the treatment combination of L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue - 70: 30) and L_5C_1, L_5C_2, L_5C_3, and L_5C_4 (Red: Green: Blue -70: 10: 20). The lowest gross return (BDT 5649000, 6516300, 7098000 and 725000) were obtained from L_1C_1, L_1C_2 L_1C_3 and L_1C_4 (White Light - Full).

4.4.2 Net return

The net returns were calculated from the difference of gross return and cost of production for different LEDs light combinations and selected microgreens crops in Table 3. The highest (BDT 6322650, 5923650, 7771650 and 7036650/ ha) and second highest (BDT 4839500.35, 4272500.35, 5217500.35 and 4839500.35/ ha) net return were obtained from the treatment combination of L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue - 70: 30) and L_5C_1, L_5C_2, L_5C_3, and L_5C_4 (Red: Green: Blue -70: 10: 20). The lowest net return (BDT 3704675, 4571975, 5153675 and 5300675) were obtained from L_1C_1, L_1C_2 L_1C_3 and L_1C_4 (White Light - Full).

4.4.3 Benefit Cost ratio (BCR)

The benefit cost ratio (BCR) was calculated from the difference of gross return and cost of production for different LEDs light combinations and selected microgreens crops in Table 3. The highest (4.14, 3.94, 4.86 and 4.5) and second highest benefit cost ratio for mustard (3.27) and radish (3.98) were obtained from L_3C_1, L_3C_2, L_3C_3, and L_3C_4 (Blue – 100%) treatment and for lettuce (3.62) and Broccoli (4.04) were obtained from L_2C_1, L_2C_2, L_2C_3, and L_2C_4 (Red – 100%) treatment. The lowest benefit cost ratio (2.55, 2.37, 2.67 and 2.55) were obtained from L_5C_1, L_5C_2, L_5C_3, and L_5C_4(Red: Green: Blue- 70:10:20) treatment.

In general, the gross and net profit earned from indoor condition was satisfactory. It can be noted that, the indoor structure is permanent. The preparation and lighting cost for five racks on 0.029m^2 box was 66500BDT (White light), 600000BDT (Red light), 666000BDT (Blue light), 707000BDT (Red: Blue) and 1733333BDT (Red: Green: Blue). This set up is installed for 10 years. This cost included in the economic analysis of the first season. However, this amount will be deducted if further cultivation is practiced in the indoor structure. In that case, the both gross and net return will increase by stated BDT.

Table 3. Cost and Return of selected microgreens under different LEDs lights in indoor condition

Treatment Combina-tion	**Cost of Production (tk/ha)**	**Yield of Microgreens (t/ha)**	**Gross return (tk/ha)**	**Net return (tk/ha)**	**Benefit cost ratio (BCR)**
L1C1	1944325	26.9	5649000	3704675	2.91
L1C2	1944325	31.03	6516300	4571975	3.35
L1C3	1944325	33.8	7098000	5153675	3.65
L1C4	1944325	34.5	7245000	5300675	3.73
L2C1	1902000	29.3	6153000	4251000	3.24
L2C2	1902000	32.8	6888000	4986000	3.62
L2C3	1902000	35.5	7455000	5553000	3.92
L2C4	1902000	36.6	7686000	5784000	4.04
L3C1	1998800	37.9	6528900	4530100	3.27
L3C2	1998800	32.8	6888000	4889200	3.45
L3C3	1998800	39.7	7959000	5960200	3.98
L3C4	1998800	34.5	7245000	5246200	3.62
L4C1	2014350	39.7	8337000	6322650	4.14
L4C2	2014350	37.9	7938000	5923650	3.94
L4C3	2014350	46.6	9786000	7771650	4.86
L4C4	2014350	43.1	9051000	7036650	4.49
L5C1	3119499.65	31.03	7959000	4839500.35	2.55
L5C2	3119499.65	35.2	7392000	4272500.35	2.37
L5C3	3119499.65	37.9	8337000	5217500.35	2.67
L5C4	3119499.65	39.7	7959000	4839500.35	2.55

Here, 1. L_1= White Light -100%, 2. L_2= Red Light- 100%, 3. L_3= Blue Light – 100%, 4. L_4= Red & Blue Light -70:30, 5. L_5= Red, Green &Blue Light- 70: 10: 20, A. C_1= Mustard, B. C_2= Lettuce, C. C_3= Radish and D. C_4= Broccoli

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted at FAB LAB (indoor structure) of Dr. Wazed Mia Research Centre in Sher-e-Bangla Agricultural University, Dhaka during the period from November to December of the year 2020 to evaluate the development of microgreens produces and evaluation of growth and nutritional profile under different LEDs light combination. It was a two-factor experiment and laid out in Randomized Complete Block Design (RCBD) with three replications. The two factors are- Factor A: Factor Four different crops (C1: Mustard, C2: Lettuce, C3: Radish, C4: Broccoli) and Factor B: five different concentrations of LEDs light (L_1= White Light - 100%); (L_2= Red Light -100%); (L_3= Blue Light- 100%); (L4= Red: Blue- 70:30); (L5= Red: Green: Blue - 70:10:20). The main photosynthetic photon flux was provided by the set of white, red, blue, red and blue, and red, green and blue light with 150, 81, 224, 248 to 89 µmol.$m^{-2.}$ s^{-1} respectively and duration was 16 hours per day. Data on different growth, yield contributing parameters and yield parameters and quality parameters were recorded and statistically analysed using MSTAT-C computer package program. Different treatments showed significant influence on most of the growth, yield contributing parameters and yield and quality parameters of mustard, lettuce, radish and broccoli.

Regarding growth parameters, the highest hypocotyl length at 3 DAS for mustard, lettuce, broccoli and radish were respectively (4.57cm, 2.97cm, 6.47cm and 3.57cm) was recorded from the treatment L_4C_1, $L_4C_{2,}$ $L_4C_{3,}$ and L_4C_4 (Red: Blue -70:30) whereas the lowest hypocotyl length (3.13 cm, 2.1 cm, 4.63 cm and 2.03 cm) was found from the control treatment L_1C_1, L_1C_2 L_1C_3 and L_1C_4 (White Light - Full). At 6 DAS the highest hypocotyl length was (7.83cm, 5.23cm, 11cm and 7.47cm respectively) from the treatment L_4C_1, $L_4C_{2,}$ $L_4C_{3,}$ and L_4C_4 (Red: Blue – 70:30) and lowest hypocotyl length was recorded (5.57 cm, 3.7 cm, 7.87 cm and 5.07 cm respectively) from the control treatment L_1C_1, L_1C_2 L_1C_3 and L_1C_4 (White Light - Full). Again, at 12 DAS the highest hypocotyl length (10.53cm, 8.47cm, 15.23cm and 11.43cm) from the treatment L_4C_1, $L_4C_{2,}$ $L_4C_{3,}$ and L_4C_4 (Red: Blue – 70:30) whereas the lowest hypocotyl length (7.67 cm, 5.53 cm, 11.2 cm and 7.73 cm) was found from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White Light - Full).

Regarding yield contributing and yield parameters, the highest fresh weight of mustard (100g), lettuce (100g), broccoli (100g) and radish (100g) were achieved from the treatment L_4C_1, $L_4C_{2,}$ $L_4C_{3,}$ and L_4C_4 (Red: Blue - 70:30) whereas the lowest fresh weight plant for mustard (72g), lettuce (92.33g), broccoli (93.3g) and radish (91.33g). Significant variation was recorded on Dry weight of mustard, lettuce, broccoli and radish as affected by different

LED-light spectral ratios. The highest total yield (0.115 kg, 0.110kg, 0.135kg and 0.125kg) were found from the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue -70:30) whereas the lowest the lowest total yield (0.078 kg, 0.090kg, 0.098kg and 0.1kg) were recorded from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White – Full). The Results revealed that the highest dry weight of mustard (4.33g), lettuce (3.5g), broccoli (4.6g) and radish (5.63g) were achieved from the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue – 70:30) whereas the lowest dry weight plant for mustard (1.367g), lettuce (1.233g), broccoli (2.63g) and radish (2.6g) were recorded from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White Light – Full) were recorded from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White Light – Full) .

In respect of quality parameters, the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue – 70:30) gave the highest SPAD value mustard (38.2nm), lettuce (16.9nm), broccoli (55.3nm) and radish (49.9nm) whereas the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White Light - Full) showed lowest SPAD value mustard (29.5nm), lettuce (12nm), broccoli (41.4nm) and radish (40.6nm). Similarly, the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue - 70:30) showed the highest nitrogen content for mustard (38.23%), lettuce (16.93%), radish (55.27%) and broccoli (93.93%) whereas the lowest nitrogen content for mustard (29.5%), lettuce (12%), radish (41.43%) and broccoli (40.6%) were recorded from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White Light - Full). Again, the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue – 70:30) gave the maximum potassium content of mustard (0.19%), lettuce (0.19%), radish (0.22%) and broccoli (0.16%) whereas the lowest nitrogen content for mustard (0.09%), lettuce (0.11%), radish (0.14%) and broccoli (0.1%) were recorded from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White Light - Full). Similarly, the maximum antioxidant capacity of mustard (0.22%), lettuce (0.23%), radish (0.19%) and broccoli (0.18%) were recorded from the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue – 70:30) whereas the lowest antioxidant capacity for mustard (0.14%), lettuce (0.14%), radish (0.11%) and broccoli (0.09%) were recorded from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White Light - Full). Finally, brix content (°Brix) was maximum in mustard (4.6%), lettuce (4.4%), radish (6.17%) and broccoli (3.83%) were recorded from the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue – 70:30) whereas the lowest °Brix for mustard (2.97%), lettuce (2.67%), radish (3.1%) and broccoli (3.27%) were recorded from the control treatment L_1C_1, L_1C_2, L_1C_3 and L_1C_4 (White Light - Full). The highest (BDT 8337000, 7938000, 9786000 and 9051000/ ha) gross return were obtained from the treatment combination of L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue - 70: 30) and the lowest gross return (BDT 5649000, 6516300, 7098000 and 725000) were obtained from L_1C_1, L_1C_2 L_1C_3 and L_1C_4 (White Light - Full). The highest (4.14, 3.94, 4.86 and 4.5) and the lowest benefit cost ratio (2.55, 2.37, 2.67 and 2.55) were obtained from L_5C_1, L_5C_2, L_5C_3, and L_5C_4(Red: Green: Blue- 70:10:20) treatment.

Conclusion

From the above results, it can be concluded that among the treatments of different LED-light spectrum in vertical farming, the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue – 70:30) had best significant positive effect on growth, yield contributing parameters and yield and quality parameters of mustard, lettuce, radish and broccoli microgreens. This treatment resulted highest fresh weight plant compared to all other treatments and the highest photosynthetic performance was found in treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue – 70:30) where SPAD value was lowest in mustard (38.2nm), lettuce (16.9nm), broccoli (55.3nm) and radish (49.9nm). Then the nutritional quality that is nitrogen content, potassium content, antioxidant capacity and (°Brix) was highest in the treatment of L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue -70:30). All of these were my objectives that I found. So, the treatment L_4C_1, L_4C_2, L_4C_3, and L_4C_4 (Red: Blue – 70:30) can be considered as the best treatment among all the treatments.

Recommendation

Considering this situation from the present study, further studies in the following areas may be suggested:

1. Some other LED-light spectrum treatments may be used in future study.
2. Another crops of microgreens and/or other vegetables need to be considered before final recommendation.

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APPENDICES

Appendix I. Agro-Ecological Zone of Bangladesh showing the experimental location

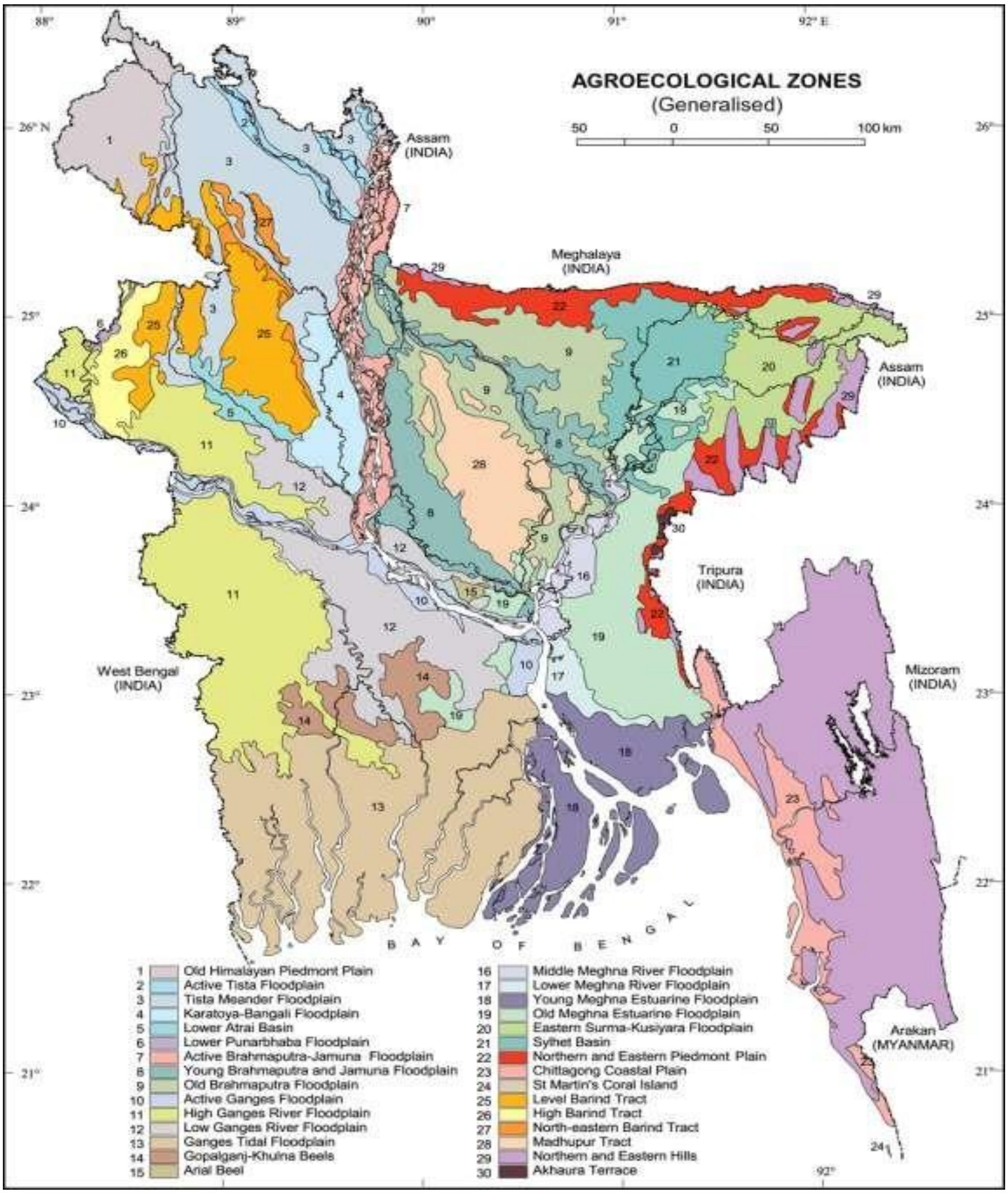

Figure 5. Experimental site

Appendix II. Monthly average temperature, relative humidity and total rainfall and sunshine of the experimental site during the period from November, 2020 to December, 2020

Month	**Air temperature (ºc)**		**Relative humidity (%)**	**Rainfall (mm) (total)**	**Sunshine (hr)**
	Maximum	**Minimum**			
November, 2020	31	18.0	99	227	5.8
December, 2020	32.4	16.3	69	0	7.9

Source: Bangladesh Meteorological Department (Climate and Weather Division), Agargoan, Dhaka – 1207

Appendix III. Analysis of variance of hypocotyl length of four different crops at different Days After Sowing (DAS) as influenced by combination of LEDs light treatment

Source of Variation	**Degree of Freedom**	**Mean Square of Hypocotyl Length**			
		3 DAS	**6 DAS**	**9 DAS**	**12 DAS**
Replication	2	4.20	10.96	2.86	5.70
Light	4	3.67**	9.79**	14.39**	20.23**
Crops	3	29.89**	61.02**	89.95**	91.08**
Light × Crops	12	0.17^{NS}	0.44^{NS}	0.50^{NS}	0.49^{NS}
Error	38	0.25	0.48	0.61	0.84
CV %		13.66	10.84	9.47	9.90

NS = Non-significant * = Significant at 5% level ** = Significant at 1% level

Appendix IV. Analysis of variance of fresh weight and dry weight for four different crops at final harvesting as influenced by combination of LEDs light treatment

Source of Variation	**Degree of Freedom**	**Mean Square of Yield contributing parameters**	
		Fresh Weight	**Dry Weight**
Replication	2	238.32	0.96
Light	4	382.94*	11.80**
Crops	3	478.00*	11.66**
Light × Crops	12	151.60^{NS}	0.76**
Error	38	83.04	0.05
CV %		9.81	7.57

NS = Non-significant * = Significant at 5% level ** = Significant at 1% level

Appendix V. Analysis of variance of quality parameters of four different crops at final harvesting as influenced by combination of LEDs light treatment

Source of Variati-on	**Degree of Freedom**	**Mean Square of quality parameters**				
		SPAD value(nm)	**Nitrogen (%)**	**Potassium (%)**	**Antioxidant (%)**	**°Bx**
Replic-ation	2	172.06	0.31	0.00	0.00	0.03
Light	4	147.03**	146.14**	0.01**	0.01**	5.43**
Crops	3	3492.68**	121.73**	0.01**	0.01**	1.57**
Light × Crops	12	6.13^{NS}	4.27**	0.00*	0.00^{NS}	0.61****
Error	38	9.04	0.07	0.00	0.00	0.02
CV %		8.52	0.56	6.82	6.95	3.70

NS = Non-significant *= Significant at 5% level**= Significant at 1% level

Appendix VI. Cost of production of selected four different microgreens under different combination of LEDs light treatment

Input Cost

Treatment Combin-ation	Labour Cost (tk.)	Seed Cost (tk)	Nutrient Cost (tk)	Light Cost (tk)	Operati-onal Cost(tk)	Total Input Cost(tk)
L1C1	480000	10000	1000000	666500	225000	74075
L1C2	480000	10000	1000000	666500	225000	74075
L1C3	480000	10000	1000000	666500	225000	74075
L1C4	480000	10000	1000000	666500	225000	74075
L2C1	480000	10000	1000000	600000	250000	72000
L2C2	480000	10000	1000000	600000	250000	72000
L2C3	480000	10000	1000000	600000	250000	72000
L2C4	480000	10000	1000000	600000	250000	72000
L3C1	480000	10000	1000000	666000	275000	76550
L3C2	480000	10000	1000000	666000	275000	76550
L3C3	480000	10000	1000000	666000	275000	76550
L3C4	480000	10000	1000000	666000	275000	76550
L4C1	480000	10000	1000000	707000	250000	77350
L4C2	480000	10000	1000000	707000	250000	77350
L4C3	480000	10000	1000000	707000	250000	77350
L4C4	480000	10000	1000000	707000	250000	77350
L5C1	480000	10000	1000000	1733333	275000	129917
L5C2	480000	10000	1000000	1733333	275000	129917
L5C3	480000	10000	1000000	1733333	275000	129917
L5C4	480000	10000	1000000	1733333	275000	129917

A. Overhead Cost

Treatm-ent Combin-ation	Cost of lease of land for 6 months (13% of value of land Tk. 45, 00000/ Year	Misce-llaneous cost (Tk. 5% of the input cost)	Interest on running capital for 1 year (Tk. 13% of cost 10,00000/year)	Subtotal (B)	Total cost of production (Tk./ha) [Input cost(A)+ overhead cost(B)]
L1C1	292500	105325	65000	462825	1944325
L1C2	292500	105325	65000	462825	1944325
L1C3	292500	105325	65000	462825	1944325
L1C4	292500	105325	65000	462825	1944325
L2C1	292500	104500	65000	462000	1902000
L2C2	292500	104500	65000	462000	1902000
L2C3	292500	104500	65000	462000	1902000
L2C4	292500	104500	65000	462000	1902000
L3C1	292500	110300	65000	467800	1998800
L3C2	292500	110300	65000	467800	1998800
L3C3	292500	110300	65000	467800	1998800
L3C4	292500	110300	65000	467800	1998800
L4C1	292500	109850	65000	467350	2014350
L4C2	292500	109850	65000	467350	2014350
L4C3	292500	109850	65000	467350	2014350
L4C4	292500	109850	65000	467350	2014350
L5C1	292500	163667	65000	521167	3119499
L5C2	292500	163667	65000	521167	3119499
L5C3	292500	163667	65000	521167	3119499
L5C4	292500	163667	65000	521167	3119499

A

B

C

D

E

Plate 9. Pictorial presentation on working in lab to perform different physical and chemical analysis: A. Recording SPAD value B. Titration C. Dry weight D. Dry sample E. Sample placing in spectrophotometer for antioxidant measurement (%).