YIELD PERFORMANCE OF THREE LETTUCE VARIETIES GROWN UNDER DIFFERENT LED LIGHT SPECTRUM

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CERTIFICATE

This is to certify that the thesis entitled

"YIELD PERFORMANCE OF THREE LETTUCE VARIETIES GROWN UNDER DIFFERENT LED LIGHT SPECTRUM"

*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 **MASTERS OF SCIENCE (M.S.)** in **HORTICULTURE**, embodies the result of a piece of bona fide research work carried out by **ZERIN TASNIM**, Registration No. **19-010208** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Dated: December, 2021

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Dedicated to

My

Beloved Parents

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The Authoress

YIELD PERFORMANCE OF THREE LETTUCE VARIETIES GROWN UNDER DIFFERENT LED LIGHT SPECTRUM

ABSTRACT

This present study was conducted to evaluate the yield performance of lettuce under led light spectrum. The experiment was comprised of two factors, *viz.,* three light spectrums [WhC - White Light (Control), Wr-Florescent Light (known as warn light), Rb- R4:B1] and three lettuce varieties [BL1- BARI Lettuce-1, RL- Red Lettuce, IcB-Ice Berg Lettuce] grown in deep water culture with a floating raft system using hydroponics nutrient solutions. Plants were hydroponically cultured with a 16-h photoperiod at 20/18 C (day/night), 75-80% relative humidity, and 100 mol m^{-2} s^{-1} photon flux density (PFD) under Rb LED, Warm (Wr) LED, and White (WhC) inside growth chambers for 42 days. The marketable sensory characteristics (crispness, sweetness, sourness, bitterness, and appearance) of fresh plants were also evaluated. The shoot and root fresh and dry weights as well as the acceptance of the plants treated with Rb were higher with IcB than those of plants treated with WhC and Wr light. Anthocyanin and antioxidant% were significantly higher in plants grown under WhCRL (9.99) and (60.54%) treatments, respectively, than in those grown under Rb treatment, whereas 0brix and Vitamin C content were higher in plants grown under Wr (6.70) and Rb (1.20) treatments, respectively, for Ice Berg Lettuce. RbIcB (0.16kg, 0.80kg, 47.35 ton) outperformed RbRL (0.14kg, 0.73kg, 43.23 ton) and WrBL1 (0.14kg, 0.73kg, 43.17 ton) in terms of fresh weight $plant^{-1}$ (kg), yield pot^{-1} (kg), and yield ha^{-1} (ton). The treatment (RbIcB) Rb light and Ice berg varieties had the best significant positive effect on growth, yield contributing parameters, and yield and quality parameters of lettuce and resulted in the highest fresh weight $plant^{-1}$ (0.16 kg) and total yield (47.35 t ha^{-1}) compared to all other treatments. Precise control of irradiance and wavelength may hold promise for improving the economic efficiency, quality, and nutritional potential of vegetables grown in controlled environments.

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ABBREVIATIONS AND ACRONYMS

AEZ	=	Agroecological Zone
BBS	=	Bangladesh Bureau of Statistics
BCSRI	=	Bangladesh Council of Scientific Research Institute
cm	=	Centimeter
CV %	=	Percent Coefficient of Variation
DAS	=	Days After Sowing
DMRT	=	Duncan’s Multiple Range Test
et al.,	=	And others
e.g.	=	exempli gratia (L), for example
etc.	=	Etcetera
FAO	=	Food and Agricultural Organization
gm	=	Gram (s)
i.e.	=	id est (L), that is
kg	=	Kilogram (s)
LSD	=	Least Significant Difference
m^2	=	Meter squares
ml	=	Milliliter
M.S.	=	Master of Science
SAU	=	Sher-e-Bangla Agricultural University
var.	=	Variety
^oC	=	Degree Celceous
%	=	Per cent
PPFD	=	Photosynthetic Photon Flux Density
GM	=	Geometric mean
mg	=	Miligram
HCl	=	Hydrochloric Acid
mg	=	Mili gram
FW	=	Fresh Weight
l	=	Litre
µg	=	Microgram
USA	=	United States of America
WHO	=	World Health Organization

CHAPTER I

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is member of Compositae family. It produces a cluster of leaves on a short stem. The leaves are delicate, crispy in texture and slightly bitter in fresh condition. They are consumed for a sweet-sour milky juice. In fact, it is globally the most requested crop for mixed salads (Fallovo, 2009) due to its high nutritive value. Generally, lettuce is low in calories, fat and sodium (Work, 1997) but rich in fiber, iron, folate, and vitamin C (Abu-Rayyan et al., 2004). It is also a good source of various other health-beneficial (anti-inflammatory, sedative, cholesterol-lowering, and anti-diabetic) bioactive compounds (Yakoot et al., 2011). Rapid population growth and limitation of cultivated land, arable land with light supplement become demandable technical practice to meet the food demand and protection to horticultural crops. Light sources such as fluorescent, metal-halide, high-pressure sodium, and incandescent lamps are generally used for plant cultivation. These sources are applied to increase photosynthetic photon flux levels but contain unnecessary wavelengths that are located outside the photosynthetically active radiation spectrum, and are of low quality for promoting growth (Kim et al., 2004a). Spectral light changes evoke different morphogenetic and photosynthetic responses that can vary among different plant species. Such photo responses are of practical importance in recent plant cultivation technologies, since the feasibility of tailoring illumination spectra purposefully enables one to control plant growth, development, and nutritional quality.

Plant development is strongly influenced by the light quality, which refers to the color or wavelength reaching a plant's surface (Johkan et al., 2010). Red (R) and blue (B) lights have the greatest impact on plant growth because they are the major energy sources for photosynthetic CO_2 assimilation in plants. Past studies examined the action spectra for photosynthesis of higher plants. It is well known that action spectra have action maxima in the B and R ranges (Cosgrove, 1981; Kasajima et al., 2008). Combined RB LED lights were proven to be an effective lighting source for producing many plant species, including lettuce, in controlled environments (Brown et al., 1995; Yanagi et al., 1996; Tanaka et al., 1998; Yorio et al., 2001; Hanyu and Shoji, 2002; Lian et al., 2002; Nhut et al., 2003; Dougher and Bugbee, 2004; Kim et al., 2004b; Lee et al., 2007; Shin et al., 2008). Furthermore, the experimental results may have been influenced in part by differences in the light intensity, and this often presents a problem when comparing results from experiments conducted under inconsistent light parameters. While it is widely understood that light intensity can positively affect photochemical accumulation (Li and Kubota, 2009; Fu et al., 2012), the effects of light quality are more complex, and mixed results were often reported. In order to apply the findings to lettuce quality and production, it is considered its importance to investigate the light-quality effects when provided as supplemental light also the sole source of light. In this regard, to identify the amounts of supplemental red LEDs, blue LEDs, or both were examined in sweet basil, strawberry, begonia seedlings, geranium seedlings, petunia seedlings, and snapdragon seedlings (Park and Runkle, 2018; Piovene *et al*., 2015). The LEDs with an R:B ratio of 0.7 improved growth and nutraceutical properties in sweet basil and strawberry (Piovene *et al*., 2015). White LEDs had similar impacts on seedling growth and electric energy consumption of artificial lights compared with mixture of red and blue lights (Park and Runkle, 2018). Chen *et al*. (2016) inferred that lettuce yield would be higher with larger red-light fraction when white light was applied as background light.

Therefore, the objectives of this study were to figure out the improvement of plant growth, nutrient of lettuce under different LED-light spectrum in vertical farming created by white, warm, red and blue light combination from sole-source of LEDs by investigating growth, yield and quality parameters of lettuce with the following objectives:

1. To investigate the growth, yield and qualitative attributes of three lettuce varieties under different LED light spectrum.
2. To find out the optimum LED light spectrum combination with lettuce varieties for growth, yield, and qualitative attributes.

CHAPTER II

REVIEW OF LITERATURE

Improvement of plant growth and yield using different LED-light spectrum is a new idea of vegetable farming. Among different LED-light spectrum treatments, lettuce has been grown to find out the best option using LED-light spectrum. Very limited studies have been performed in these aspects where in Bangladesh this the first research on it. Some of the recent past information on the improvement of plant growth, yield and photosynthesis activity of lettuce under different LED-light spectrum in vertical farming have been reviewed below:

According to (Specht *et al*., 2014). vertical farming facilitates the production of high value crops with higher yield than obtained from conventional farming by efficient utilization of resources such as water, nutrients, space and time, thereby, reducing carbon footprint. Vertical farming technology does not require huge arable land to produce crops and thus is agriculturally independent. This innovation utilizes both the horizontal and vertical spaces 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 farming 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 farming 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 (Specht *et al*., 2014).

Nwosisi et al., (2017) conducted a study on cultivar trials in organic vertical system on the certified organic farm of the Tennessee State University, Nashville. Several cultivars of lettuce, chard, kale, mustard-green, basil and coriander were grown in the vertical-grow system and were harvested successfully. Automated system called 'Drip Organics' provided organic nutrients to the plants. Perlite and coco fiber media was used in the stacked pots. It was concluded that although modern organic farming would remain a widely used food production method as evidenced by its global acceptance and steady growth, organic vertical farming would have a potential to provide food security, year-round produce and ease transportation of food within urban and semi urban areas.

Al-Chalabi (2015) prepared a paper with the purpose to examine the feasibility and plausibility of the vertical farming concept from socio technical, mixed methods, research perspective. It included (1) examining energy requirement to power such a building and probability of renewable energy to meet the onsite demands of the building by constructing an energy model, (2) quantifying the carbon footprint of vertically grown produce and subsequently comparing that to conventionally grown produce, and (3) conducting interviews to explore how relevant stakeholders perceive the concept of vertical farming in order to identify the barriers and opportunities towards possible uptake of the technology. The findings indicated that vertical farming could be used as a tool to supply food to cities in a sustainable manner depending on the location and design.

Banerjee and Adenaeuer (2013) carried out a study to construct a Vertical Farm and thereof investigate the economic feasibility of it. In a concurrent Engineering Study initiated by DLR Bremen, a farm, 37 floors high, was designed and simulated in Berlin to estimate the cost of production and market potential of this technology. It yielded about 3,500 tons of fruits and vegetables and 140 tons of tilapia fillets, 516 times more than expected from a footprint area of 0.25 ha due to stacking and multiple harvests. The investment costs added up to € 200 million, and it required 80 million liters of water and 3.5 GWh of power per year. The produced food ranged between € 3.50 and € 4.00 per kilogram. In view of its feasibility, they estimated a market for about 50 farms in the short term and almost 3000 farms in the long term.

An experiment about vertical garden was done at the Centre for Land Resources, Gadjah Mada University, Yogyakarta, Indonesia by Utami and Jayadi (2012). Raised beds (shelf, a place holder) or growing beds were the basic unit of an intensive garden. Several of these beds were made with 6 levels of 6×4 m2 and 4 levels of 4×1.72 m2 . Nutrients were supplied by organic matter (manure and compost), while water was supplied as treated wastewater. Then several vegetables (Chinese cabbage, lettuce, water spinach, chili red) and fruits (lemon, guava, mango, passion fruit) were planted. The results showed that vertical gardening were best suited for plants that required maximum sunlight such as fruit and also several vegetables. Plants grown in a vertical garden were less accessible to diseases and pests, and crop harvesting and cultivation was easier. Vertical gardening provided adequate aeration to the plants and also increased the beauty of the garden. Overall, the yield of vertical gardening was higher than traditional plantation methods.

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 (Chen *et al*., 2019). 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*., 2017).

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

Various species exhibit different light-response modes; nevertheless, more experiments need to be conducted to ensure the application of optimal red and blue light with alternating intervals. The effects of supplementary light on growth and health-promoting compounds in Brassica vegetables have been reported; for example, the spectra and intensity of supplemental LED illumination were associated with the enhanced accumulation of lutein and b-carotene in Brassicaceae microgreens (Brazaityte *et al*., 2015). Both plant growth and the accumulation of health-promoting compounds in Chinese kale and pakchoi were increased in association with the supplemental blue light intensity (Li *et al*., 2019; Zheng *et al*., 2018).

Light is one of the most important environmental factors that influence plant growth and development. Terrestrial sunlight consists of ultraviolet (UV), visible light and infrared radiation, in which visible light accounts for almost half of the absorption spectrum (Abe, R. 2010). The wavelength of the UV radiation lies in the range of 100–400 nm, visible light in the range of 400–700 nm and infrared in the range of 700–1000 nm. Even though the terrestrial sunlight spectrum is wide, plants can only utilize the visible light spectrum as the sole source of energy for photosynthesis, and this narrow spectrum of electromagnetic radiation is defined as photosynthetically active radiation (PAR) (McCree, 1971, 1972). Interestingly, plants can sense and detect variations in the light intensity and spectral composition of their native environment to adjust their growth and developmental processes (Fiorucci and Fankhauser, 2017). This has given rise to various plant responses such as photomorphogenesis, photoperiodism and phototropism (Kendrick and Kronenberg, 2012; Whippo and Hangarter, 2006). Photomorphogenesis 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 (Wong *et al*., 2020).

Plants use photosynthetic pigments in their leaves to capture energy from PAR to drive synthesis of sugar molecules. These photosynthetic pigments are present around the thylakoid membranes of chloroplasts to serve as primary electron donors in the electron transport chain (Anderson, 1986). In plants, the most abundant photosynthetic pigments are chlorophyll a and chlorophyll b (Shoaf and Lium, 1976). 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 (Netto *et al*., 2005). 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 (Inskeep and Bloom, 1985). Due to the chemical structures of chlorophyll a and b, the absorption spectra are not uniform across PAR and they have minimal absorption in the 500–600 nm range, thus, reflecting the colors 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 (Havaux, 1998).

The development in LED lighting technology, allowing the flexible modifications of light spectra, has enabled the research and application of light quality in enhancing leafy green qualities in controlled environment for better growth, colour, flavour and phytonutrient content. The effects of LED spectra on the growth, development and metabolite accumulation of leafy vegetables have been intensively studied, especially in lettuce. However, it is challenging to extract an optimal lighting recipe from all of this research due to inconsistent experimental parameters ranging from the precise spectral composition to the length of treatment (Wong *et al*., 2020). Studies conducted to understand the different spectral composition on improving biomass and quality of leafy vegetables have focused primarily on red and blue wavelengths, the absorptance maxima of chlorophyll. Red light (RL) has the highest quantum yield, whereas blue light (BL) is considerably less efficient in driving photosynthesis (Inada, 1976; McCree, 1972).

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 (Terashima *et al*., 2009). RL induces many physiological responses including leaf development, stomatal opening, chlorophyll and carbohydrate accumulations (Azad *et al*., 2020; Hogewoning *et al*., 2010; Lee *et al*., 2016; Yang *et al*., 2016). BL influences photosynthetic activity by inducing stomatal 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*., 2016a). 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*., 2012; Matsuda *et al*., 2007). BL also regulates several plant morphogenic responses including leaf expansion and shoot elongation (Li and Kubota, 2009; Metallo *et al*., 2018).

Kim *et al*., 2004) conducted an experiment to determine the effects of LEDs on net photosynthetic rate, growth, and leaf stomata of chrysanthemum plantlets *in vitro* grown in MS medium, it was found that the net photosynthetic rate was highest under red (650 nm) and blue (440 nm) combination and lowest under blue-far red (720 nm) combination and blue. Red and red-far red combination resulted in the highest stem elongation but with stem fragility. Shoot growth excluding stem elongation was the greatest under red-blue combination and fluorescent light. When *Lactuca sativa* of variety red curly lettuce was grown under different light spectrum, it was found that anthocyanin synthesis, protein content and phenylalanine ammonia-lyase enzyme activity were highest in combined radiation of blue and red-light treatment (Heo *et al*., 2012). In another study, where red and green basil (*Ocimum basilicum*) microgreens were grown with blue and red LED, it was found that growth of microgreens was enhanced with predominantly blue illumination showing larger cotyledon area and higher fresh mass, enhanced chlorophyll a, and anthocyanin pigments contents. Stimulation of phenolic synthesis and free radical scavenging activity were improved by pre1dominantly red light in the green cultivar and blue light in the red cultivar (Lobiuc *et al*., 2017), which indicates that LED light has an influence on the colour of the leaf.

A combination of red and blue LEDs routinely used in indoor farming has a relatively higher production efficiency compared to other light sources such as fluorescent lamps with the same light irradiance (Amoozgar *et al*., 2017; Johkan *et al*., 2010; Lee *et al*., 2016). The optimal ratio between BL and RL is crucial in determining plant productivity and a low B/R ratio generally favours biomass accumulation. In a background of RL, 25% of BL (B25R75) has produced red pakchoi with greater biomass, leaf area and anthocyanin accumulation compared to white LED (Mickens *et al*., 2019), while lettuce ('Grizzly') grown under B30R70 has 75% increase in biomass when compared to those under white LED (Amoozgar *et al*., 2017). Kale grown under

B20R80 increases by 12.5% in dry mass compared to those under white light and shows significant morphological alteration with shorter and more compact plants, which is consistent with BL's roles in inhibiting extension growth (Metallo *et al*., 2018). The above-ground dry weight is almost doubled in tatsoi when RL is supplemented with 10% BL (Virsile *et al*., 2019). In Chinese kale, a 2–3 fold increase in leaf area and shoot dry weight has been reported when RL was supplemented with 18% BL (He *et al*., 2015).

Monochromatic RL environment, however, triggers abnormal plant morphology in lettuce, spinach, kale and basil that includes the development of elongated hypocotyl, long petioles and thin wide leaves with reduced chlorophyll content (Amoozgar *et al*., 2017; Johkan *et al*., 2010; Kang *et al*., 2016; Naznin *et al*., 2019). The impaired development seen in these studies resembles those associated with shade avoidance response that is triggered by low light, a high red to far-red (R/Fr) ratio, low BL or high green light levels in the environment (Wang *et al*., 2020).

Too much BL in the irradiance can also have an adverse effect on plant growth and development. When the proportion of blue LED exceeds 11% in a broad-spectrum background, dry mass and leaf area are decreased for lettuce ('Waldmann's Green'), radish and pepper (Cope *et al*., 2014). Similar parameters in two other cultivars of lettuce ('Rouxai' and 'Green Skirt') as well as kale are also negatively correlated with the amount of BL in a red background (Dou *et al*., 2020; Kang *et al*., 2016; Meng *et al*., 2020). An increase from 16 to 24% of BL leads to the reduction in the leaf area and dry weight of Chinese kale grown in a red background (He *et al*., 2015).

A lower level of irradiance does not seem to negate the effect of high fraction of BL on lettuce ('Lollo Rosso') growth as the leaf area and shoot dry weight decrease significantly as BL fraction is increased from 25% to 50% at 90 μmol $m^{-2}s^{-1}$ (equivalent to 5% of full sunlight) (Azad *et al*., 2020). Spinach seems to be more vulnerable to BL-induced negative effects compared to lettuce and komatsuna (*Brassica rapa* var.

perviridis) as severe reduction in dry weight has been observed under blue fluorescent lamp (Ohashi-Kaneko *et al*., 2007).

Green light (GL), unlike RL and BL, can penetrate deeper into a leaf (Sun *et al*., 1998) and canopy (Massa *et al*., 2015). A higher fraction of GL may thus stimulate photosynthesis deep within a leave and canopy layer, increasing whole-plant photosynthesis. Such increase in photosynthesis is not possible with excess RL or BL due to their strong absorption by chlorophyll in the upper part of the leaf (Terashima *et al*., 2009). This unique contribution of GL to photosynthesis suggests that it may be beneficial to plant growth and development when used to supplement red and blue irradiation, especially for vegetables that form thick canopy. GL can also act as a shade signal (Sellaro *et al*., 2010; Zhang *et al*., 2011) and it can antagonize a number of BL-induced responses including inhibition of extension growth (Folta, 2004) and stimulation of stomata opening (Talbott *et al*., 2002).

Yellow light (YL, 580–600 nm) is poorly absorbed by any of the photosynthetic pigments and we know relatively little about the effect of YL on photosynthesis as compared to other wavelengths. Research using green fluorescent lamp includes 500–600 nm wavelengths and therefore contains YL (Dougher and Bugbee, 2001; Kim *et al*., 2004). These coupled with the low efficiency of yellow LEDs (Jiang *et al*., 2019) could explain the scarcity of studies exploring the use of YL in the cultivation of leafy greens. Yellow wavelength (ranging from 20 to 30% of photosynthetic photon flux density (PPFD) = 200 and 500 μmol $m^{-2}s^{-1}$) from high pressure sodium lamp and metal halide lamp has been reported to inhibit growth in lettuce (‘Grand Rapids’) by suppressing chlorophyll formation (Dougher and Bugbee, 2001), while a more recent study shows minimal effect of YL (6.7% of PPFD = 300 μmol $m^{-2}s^{-1}$) on lettuce (‘Red & Green Cos’) growth and biomass production (Virsile *et al*., 2020). In another cultivar (‘Green Oakleaf’), supplemental YL (30% of PPFD = 135 μmol $m^{-2}s^{-1}$) inhibits growth compared to those under white LED (Chen *et al*., 2016). Thus, YL negatively impacts on lettuce growth at high fraction. The effect of YL on the growth of other leafy greens as well as on the accumulation of phytonutrients await further investigation. Such studies will be facilitated with the recent development of a more efficient light source (Jiang *et al*., 2019).

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; Park and Runkle, 2018). Compared with other wavelengths, red and blue lights were paid more attention to lettuce (Lee *et al*., 2010; Son and Oh, 2015; Stutte *et al*., 2009; Wang *et al*., 2016b), cucumber seedlings (Hernandez and Kubota, 2016), tomato seedlings (Hernandez *et al*., 2016; Liu *et al*., 2018), Mesembryanthemum crystallinum (He *et al*., 2017), and sweet basil (Pennisi *et al*., 2019) in past and recent decades, as red and blue lights were considered more effectively absorbed by chlorophylls of plant leaves (McCree, 1971). Moreover, plants grown under the combination of red and blue lights had bigger stem diameter (Yang *et al*., 2018), higher net photosynthetic rate (Pn) (Wang *et al*., 2016b), anthocyanin (Lee *et al*., 2010; Stutte *et al*., 2009), and arginine (Zhang *et al*., 2018a) compared with those grown under monochromatic red light. In addition, red and blue LEDs had higher photosynthetic photon efficiency (Park and Runkle, 2018). As a result, red and blue LEDs are commonly applied for commercial production in PFALs. Previous studies also investigated the suitable R:B ratio created by mixture of red and blue lights for dry weight accumulation in lettuce (Wang *et al*., 2016b), cucumber seedlings (Hernandez and Kubota, 2016), and M. crystallinum (He *et al*., 2017).

Plants grown under mixed red and blue LEDs appeared purplish, thus making visual assessment of disease symptoms and growth disorder difficult to observe (Kim *et al*., 2007). One solution for this limitation was to apply white LEDs, alone or with blue LEDs, red LEDs, or both (Park and Runkle, 2018; Yan *et al*., 2019). The world's first white LED was created in 1996 as a phosphor coating used by blue LED (Bourget, 2008) and the present white LEDs showed increased energy efficiency as the highly efficient blue-emitting diodes were invented (Pust *et al*., 2015). White LEDs were suggested as a substitute lighting source compared with fluorescent lamps for lettuce production in PFALs (Park *et al*., 2012), and green leaf lettuce grown under white LEDs had higher leaf fresh weight (Zhang *et al*., 2015), root fresh weight, and phenolic concentration (Son *et al*., 2012) than those grown under monochromatic red LEDs. Moreover, white LEDs were used as background lighting in 'Green Oak Leaf' lettuce reported by Chen *et al*. (2016), and the effects of red, green, blue, yellow, and far red lights as supplemental lights were examined. Results indicated that lettuces grown under white light combined with supplemental red light appeared vigorous and compact compared with those grown under white LEDs alone. Mickens *et al*. (2018) also observed similar results in red romaine lettuce at harvest. To identify the amounts of supplemental red lights, blue lights, or both, combination of white with different fractions of red LEDs, blue LEDs, or both were examined in sweet basil, strawberry, begonia seedlings, geranium seedlings, petunia seedlings, and snapdragon seedlings (Park and Runkle, 2018; Piovene *et al*., 2015).

Daily light integral is the total amount of light received by plants in 1 d (Bruggink and Heuvelink, 1987; Kozai *et al*., 2018). Linear relationships were often observed between DLI and biomass of lettuce (Gent, 2014; Zhang *et al*., 2018b), average shoot dry weight per average internode number of Celosia seedlings (Pramuk and Runkle, 2005), and nutritional values of sweet basil (Dou *et al*., 2018). The suitable DLIs for hydroponic lettuce production were recommended at 12.67 mol $m^{-2}d^{-1}$ (Fu *et al*., 2017) and 14.40 mol $m^{-2}d^{-1}$ (Zhang *et al*., 2018b), respectively. However, few studies examined the relationships between DLI and carbohydrate accumlation under LEDs with different R:B ratios when white LEDs were used as base lighting source, and there were few bases for the spectrum design of relatively broad and wide LEDs for lettuce cultivation. In addition, few researchers focused on the energy use efficiency for lettuce production in PFALs.

In the commercial greenhouse, light supplementation using artificial light can significantly increase crop yield and nutrition quality, especially in low-light-intensity seasons like winter and late autumn (Lu *et al*., 2012; Yorio *et al*., 2001). With the development of urban agriculture, artificial light has become the most important way to control the light conditions. For a long time, people were using fluorescent lamps, filament lamps and high-pressure sodium lamps (HPL), and much research was carried out to test their effects (Tibbitts *et al*., 1983). However, these kinds of light tend to consume large amounts of electrical energy and release a lot of heat (which will also increase the cooling system cost), and their spectra are not very suitable for plants, which leads to excessive waste of energy (Randall and Lopez, 2014).

The most important element in controlling artificial farming costs is supplying light for photosynthesis and growth by light sources with high photoelectric efficiency. Light-emitting diodes (LEDs) have been proposed as alternative light sources in controlled agricultural environments since, compared with traditional horticulture light sources (e.g., HPL), LEDs have drastic advantages, such as superior lifetime, reduced size, cooler emitting temperature, and reduced energy consumption (Massa *et al*., 2008).

An exciting potential of using LED lighting is the development of species-specific light recipes comprising the optimum proportion of specific narrow-band wavelength light that can optimize plant growth, development and other desirable traits (e.g., increase phytochemical content) (Bian *et al*., 2015, 2016), whilst significantly reducing the energy input compared with traditionally used horticulture light sources. Recently, the effect of LEDs on plant growth and development has aroused increasing interest. However, the results of related studies are sometimes different, and even contradictory (Avercheva *et al*., 2009; Bian *et al*., 2016; Hogewoning *et al*., 2010; Urbonaviciute *et al*., 2007).

Plants grown in vertical farming systems are surrounded by walls and receive no sunlight; therefore, artificial lamps provide the only light source. However, conventional light sources have drawbacks to their use, causing excessive heat on leaf surfaces and leading to undesirable effects on plant growth (Martineau *et al*., 2012). Thus, there is a need for the development of innovative artificial lighting for optimizing the light environment. Light-emitting diodes (LEDs) have seen great development with many technological advancements and have incomparable advantages, and their application to lighting in plant cultivation has increased rapidly (Xu, 2019). The price of LEDs has decreased remarkably over the past several years, and many studies have been concentrated on defining the optimal light environments to enable the high-quality, high-speed production of various plant species (Massa *et al*., 2008). This makes LED lighting systems a cost-effective solution for controlled environment agriculture systems.

Natural sunlight contains a wide continuum of wavelength and fluence and is optimal for plants (Darko *et al*., 2014). Therefore, manipulating the light conditions of artificial light sources is essential for growing plants in vertical farming to obtain electricity cost savings and balance the yield and quality of plants (Dou *et al*., 2018). It is well documented that the various regions of light spectra have different efficiencies in enhancing the plant photosynthetic process and plant morphological, physiological, and biochemical responses (Folta and Maruhnich, 2007). Within the visible light spectral range (400–700 nm), many researchers have focused on studying the role of red (R) (600–700 nm) and blue (B) (400–500 nm) light and on defining their optimal combination ratios because their wavelengths are close to the absorbance of photosynthetic pigments that effectively drive photosynthesis (Sabzalian *et al*., 2014). Many studies have confirmed the role of R LEDs in increased biomass accumulation, stem elongation, and leaf expansion, as well as the effect of B LEDs in chlorophyll production, stomata opening, and photosynthesis (Muneer *et al*., 2014). Therefore, monochromatic R or B LEDs and combined RB LEDs have been widely used in scientific research and commercial vertical farming (Ouzounis *et al*., 2015). However, plants exposed to combination R and B lights normally appear purplish-grey to the human eye, which leads to difficulties in the visual assessment of plant health (e.g., disease symptoms, nutritional deficiencies, and physiological disorders) (Kim *et al*., 2005).

Advanced LED technology enables broad-spectrum W LED light that consists of R, G, and B lights. This could be effective for use in vertical farming to improve plant growth and provide desirable lighting for human vision. Several approaches have been used to achieve W LED light. The most common and successful approach is the use of a B LED chip with phosphors to convert a part of the B light to R and G lights. The B light from the LED chip and the R and G lights converted by phosphors create W light, leading to the steady increase in the efficiency of B LEDs and consequently improving the W LED efficiency (Cope and Bugbee, 2013). W LED light can also be created by combining several LED chips that emit monochromatic R, B, and G lights (Chang *et al*., 2012), enabling control of the ratios of R, B, and G lights desirable for human vision and plant growth responses. This approach has become feasible with the highly efficient use of B and G LEDs and possesses high reliability and durability as well as low energy consumption (Pimputkar *et al*., 2009).

Different crops e.g. lettuce crops grown with red and blue LED lighting (95% red and 5% blue) used 50% less energy per unit dry biomass accumulated than under traditional light sources, which indicates that the significant reduction in energy consumption for plant-growth by using LED than traditional light sources (Poulet *et al*., 2014). In an experiment on the indoor cultivation of basil and strawberry, it was found that the plants expressed increased biomass, fruit yield, antioxidant content and reduced nitrate content 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 with improved nutraceutical properties (Piovene *et al*., 2015).

Huang *et al*. (2021) conducted a study aimed to evaluate the effects of alternating red (660 nm) and blue (460 nm) light on the growth and nutritional quality of two-leaf-color Lettuce (*Brassica campestris* L. ssp. *chinensis* var. communis). Four light treatments (supplemental alternating red and blue light with intervals of 0, 1, 2, and 4 hours, with a monochromatic light intensity of 100 µmol $m^{-2}s^{-1}$ and a cumulative lighting time of 16 hours per day) were conducted in a greenhouse under identical ambient light conditions (90 to 120 µmol m^{-2} s^{-1} at 12:00 AM) for 10 days before green- and red-leaf Lettuce were harvested. The results showed that the two-leaf color Lettuce receiving alternating red and blue light exhibited more compact canopies and wider leaves than those under the control treatment, which was attributed to the shade avoidance syndrome of plants. The present study indicated that the biomass of green-leaf Lettuce was much higher than that of red-leaf Lettuce , but the nutritional quality of green-leaf Lettuce was lower than that of red-leaf Lettuce , and seemingly indicating that the regulation of metabolism for Lettuce was species specific under light exposure.

The trends of both biomass and the soluble sugar content were highest under the 1-hour treatment. The contents of chlorophyll a and total chlorophyll in both cultivars (green- and red-leaf Lettuce) were significantly increased compared with control, without significant differences among the 1-, 2-, and 4-hour treatments, whereas chlorophyll b exhibited no significant difference in any treatment. Alternating red- and blue-light treatment significantly affected the carotenoid content, but different trends in green and red-leaf Lettuce were observed, with the highest contents being detected under the 1-hour and 4-hour treatments, respectively. With increasing time intervals, the highest soluble protein contents in two-leaf-color Lettuce were observed in the 4-hour treatment, whereas nitrate contents were significantly decreased in the 4-hour treatment. Compared with 0 hours, the contents of vitamin C, phenolic compounds, flavonoids, and anthocyanins in two-leaf-color Lettuce were significantly increased, but no significant differences were observed in vitamin C, phenolic compounds, and flavonoids among the 1-, 2-, and 4-hour treatments, similar to what was found for the anthocyanin content of green-leaf Lettuce . However, the content of anthocyanins in red-leaf Lettuce gradually increased with increasing time intervals, with the highest content being found in the 4-hour treatment. Supplemental alternating red and blue light slightly increased the antioxidant capacity [1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging rate and antioxidant power], but no significant differences were observed after 1, 2, and 4 hours of treatment. Taken together, treatment with an interval of 1 hour was the most effective for increasing the biomass of Lettuce in this study, but treatment with a 4-hour interval should be considered to enhance the accumulation of health-promoting compounds.

Nguyen *et al*. (2021) carried out a study aimed to examine the effect of W LED light sources on the growth and quality of butterhead and romaine lettuce and reported that white (W) light-emitting diode (LED) light has been used as an efficient light source for commercial plant cultivation in vertical farming. Three W LED light sources including normal W light (NWL) which has 450 nm as its pumping wavelength and two specific W lights (SWL1 and SWL2) with shorter blue peak wavelength (437 nm) were used to grow lettuce in comparison to a red (R) and blue (B) LED combination. As a result, SWL1 and SWL2 treatments with the same electrical power or photosynthetic photon flux density (PPFD) resulted in more growth of both lettuce cultivars compared to RB treatment. Some phenolic and flavonol contents were increased in the RB treatment, whereas SWL2 treatment stimulated the accumulation of other phenolic and flavonol compounds. Meanwhile, neither NWL nor SWL1 treatments increased the individual phenolic and flavonol contents in either cultivar (except for some flavonols in romaine lettuce in the SWL1 group). In addition, light and energy use efficiencies were also highest in the SWL1 and SWL2 treatments. These results illustrate the positive effects of specific W LED light on lettuce growth and quality, and suggest that the specific W LED light sources, especially SWL2, could be preferably used in vertical farming.

Agricultural production in controlled indoor farming offers a reliable alternative to food and nutrition supply for densely populated cities and contributes to addressing the impending food insecurity. Leafy vegetables, rich in vitamins, minerals, fibres and antioxidants, account for over half of the indoor farming operations worldwide. Light is the foremost environmental factor for plant growth and development, and the success of indoor farming largely depends on lighting qualities. The energy efficient light-emitting diode (LED) has been increasingly used in indoor farming systems. A study was carried out by Wong (2020) for seeing the lights for leafy greens in indoor vertical farming and was provided an updated overview of the current indoor vertical farming systems, the mechanisms of light perception by photoreceptors, and the effects of LED spectra or intensity on growth and phytonutrient accumulation of leafy greens for this study. It was reported that by Wong (2020) that lighting quality and quantity can be manipulated to improve yield and phytonutrient contents of leafy greens. As responses of leafy greens to light are dependent on genotype and developmental stage, light recipe targeting different developmental stages should be formulated for different species for maximizing yield. While it has been known that blue wavelength has a more prominent positive impact on phytonutrient accumulation than red, little is known for other wavelengths. Moreover, recent findings that green wavelength inhibits plant growth in a blue-wavelength-dependent manner highlight the need for future research to investigate interactive effects of different wavelengths on modulating plant growth and metabolism.

Yan *et al*. (2020) reported that red plus blue light-emitting diodes (LEDs) are commonly applied in plant factories with artificial lighting due to photosynthetic pigments, which absorb strongly in red and blue light regions of the spectrum. However, plants grown under natural environment are used to utilizing broad-wide spectrum by long-term evolution. In order to examine the effects of addition light added in red plus blue LEDs or white LEDs, green and purple leaf lettuces (Lactuca sativa L. cv. Lvdie and Ziya) were hydroponically cultivated for 20 days under white LEDs, white plus red LEDs, red plus blue LEDs, and red plus blue LEDs supplemented with ultraviolet, green or far-red light, respectively. The results indicated that the addition of far-red light in red plus blue LEDs increased leaf fresh and dry weights of green leaf lettuce by 28% and 34%, respectively. Addition of ultraviolet light did not induce any differences in growth and energy use efficiency in both lettuce cultivars, while supplementing green light with red plus blue LEDs reduced the vitamin C content of green leaf lettuce by 44% and anthocyanin content of purple leaf lettuce by 30% compared with red plus blue LEDs, respectively. Spectral absorbencies of purple leaf lettuce grown under red plus blue LEDs supplemented with green light were lower in green light region compared with those grown under red plus blue LEDs, which was associated with anthocyanin contents. White plus red LEDs significantly increased leaf fresh and dry weights of purple leaf lettuce by 25%, and no significant differences were observed in vitamin C and nitrate contents compared with white LEDs. Fresh weight, light and electrical energy use efficiencies of hydroponic green and purple leaf lettuces grown under white plus red LEDs were higher or no significant differences compared with those grown under red plus blue LEDs. In conclusion, white plus red LEDs were suggested to substitute for red plus blue LEDs in hydroponic lettuce (cv. Lvdie and Ziya) production in plant factories with artificial lighting.

Yan and He (2019) conducted a study to investigate the effects of daily light integrals (DLI) and light emitting diodes (LEDs) light quality (LQ) on growth, nutritional quality, and energy use efficiency of hydroponic lettuce (*Lactuca sativa* L.) in a plant factory with artificial lighting (PFAL). Hydroponic lettuce plants (cv. Ziwei) were grown for 20 days under 20 combinations of five levels of DLIs at 5.04, 7.56, 10.08, 12.60, and 15.12 mol m^{-2} d^{-1} and four LQs: two kinds of white LEDs with red to blue ratio (R:B ratio) of 0.9 and 1.8, and two white LEDs plus red chips with R:B ratio of 2.7 and 3.6, respectively. Results showed that leaf and root weights and power consumption based on fresh and dry weights increased linearly with increasing DLI, and light and electrical energy use efficiency (LUE and EUE) decreased linearly as DLI increased. However, no statistically significant differences were found in leaf fresh and dry weights and nitrate and vitamin C contents between DLI at 12.60 and 15.12 mol $m^{-2}d^{-1}$. Also, no effects of LQ on leaf dry weight of hydroponic lettuce were observed at a DLI of 5.04 mol $m^{-2}d^{-1}$. White plus red LEDs with an R:B ratio of 2.7 resulted in higher leaf fresh weight than the two white LEDs. LUE increased by more than 20% when red light fraction increased from 24.2% to 48.6%. In summary, white plus red LEDs with an R:B ratio of 2.7 at DLI at 12.60 mol $m^{-2}d^{-1}$ were recommended for commercial hydroponic lettuce (cv. Ziwei) production in PFALs.

Manawasinghe and Weerasekara (2020) carried out a research and examined the plant growth and yield of vertically grown pakchoi (*Brassica rapa* var. *chinensis*) (in nutrient film technique (NFT) culture) under supplementary lighting with two different combinations of blue to red color LEDs (1:9 and 1:2 ratios) in comparison with horticulture grade and non-horticulture grade (recommended for general use) white (full spectrum) LED while keeping sunlight as the control treatment. Meanwhile NFT culture was compared to plant growth, yield and nitrate accumulation of basil (*Ocimum basilicum* L.) in comparison with conventional soil, culture and compost mixed cocopeat substrate in a replicated trial, conducted under greenhouse conditions with intensive micro climate control. A significantly high vegetative growth and total to yield could be found in the NFT grown basil. The nitrate accumulation in basil leaves was well below the maximum permissible limit (MPL), set-fourth by the recommendations of the European Health Commission. Meanwhile, the highest overall leaf quality of pakchoi was achieved by the normal LEDs. Horticulture graded to LED maintained fairly high chlorophyll a and b contents contributing to its characteristic leaf color.

Bian *et al*. (2017) carried out a study and found that light supplementation can increase crop yield in greenhouses by promoting photosynthesis and plant growth. However, the high energy costs associated with light supplementation are a predominant factor that limits development and profit improvement of controlled environment agriculture. Light-emitting diodes (LEDs) are a promising technology that has tremendous potential to improve irradiance efficiency and to replace traditionally used horticultural lighting. Compared with traditional light sources (e.g., high-pressure sodium lamps and metal halide lamps) used in crop production, LEDs have distinct advantages, such as their small size, long lifetime and high photoelectric conversion efficiency. Most importantly, as a monochromatic light source, the spectrum of LEDs can be adjusted based on plant growth requirements. This project aimed to investigate energy-use efficiency, vegetable nutrition and photosynthesis improvement of light supplementation in a protected horticulture system. In the initial phase, the effects of LED light on plant growth and light-use efficiency for pakchoi and photosynthetic performance were investigated. The results showed 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. Monochromatic red and blue light LEDs resulted in significant decreases in Pn of tomato plants, but the stomatal conductance (Gs) for monochromatic blue LEDs was higher than that for FL. 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

The present research work was conducted at the indoor condition of Dr. M. Wazed Miah Research Centre, Sher-e-Bangla Agricultural University, Dhaka during the period from July 2020 to October 2020. Brief descriptions of materials and methods that are used in carrying out the experiment have been presented in this chapter.

3.1 Experimental site

The experimental site is located at 90°22′E longitude and 23°4l′N latitude at an altitude of 8.6 meters above the sea level. The experimental site is presented in (Appendix I).

3.2 Climatic condition

The experimental area is under the sub-tropical climate that is characterized by less rainfall associated with moderately low temperature during rabi season, (October-March) and high temperature, high humidity and heavy rainfall with occasional gusty winds during kharif season (April-September). Details of weather data in respect of temperature (^{0}C), rainfall (mm) and relative humidity (%) for the study period was collected from Bangladesh Meteorological Department, Agargoan, Dhaka-1207 and presented in (Appendix II).

3.3 Planting material used for the experiment

Seeds of the Lettuce variety BARI Lettue-1, Red Fire and Ice Berg was used for the experiment. The seeds were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur and local market in Dhaka, Bangladesh.

3.4 Treatments of the experiment

Two factorial experiments consisting three treatments each of factor as follows:

Factor A: Light Spectrum

1. WhC-White Light (Control) [100 $\mu mol \cdot m^{-2} \cdot s^{-1}$]
2. Wr - Fluorescent Light (known as Warn light) [100 $\mu mol \cdot m^{-2} \cdot s^{-1}$]
3. Rb - Red and Blue (R4:B1) [100 $\mu mol \cdot m^{-2} \cdot s^{-1}$]

Factor B: Lettuce Variety

a. BL1- BARI Lettuce-1
b. RL- Red Lettuce (Red Fire Lettuce)
c. IcB- Ice Berg Lettuce

3.5 Design and layout of the experiments

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. Two factors (LED light spectrum) and (Variety) was considered for the present study. The layout of experiment field is presented in (Appendix III)

3.6 Details of the experimental operations

The particulars of the experimental operations carried out during the experiment are presented below:

3.6.1 Seed sowing and growing of seedlings

For germination, the first seeds of BARI Lettue-1, Red Fire, and Ice Berg were taken into a bowl full of water to select viable seeds that were lying on the bottom of the bowl. Due to the help of germination by the breaking of seedcoat, all the selected seeds were taken into water for 1 hr. On the other hand, germination media and the indoor room by controlling temperature and humidity were prepared. Rockwool cubes as germination media were used in order to increase the area of the clear germination box with a small cube-shaped unit (Plate 1). By placing the cubes into the clear box, germination media is prepared. Then sow 2/3 seeds per pit on the rockwool cubes. Then these cubes were covered with some heavy materials (one box upon another) to warm the area, which easily causes germination. All of these boxes were taken under warm room conditions. Some water was also offered to keep the media moisturized. The moisture content of the media was determined by pressing it with a finger.

3.6.2 Preparation of transplanting media

After 3-5 days, the seedlings emerge. For going into a matured seedling stage and also for hardening purposes, the seedlings were kept in this germination media under warm conditions. At the same time, the final media, such as transplanting media, started to be made (Plate 2). This research is totally based on hydroponic solutions and was performed indoors. Several racks (3) were prepared in vertical conditions and hydroponic solutions were prepared by HRC BARI.

In each rack, 3 solution boxes (25L) as well as 3 replications were placed. For lighting purposes, different colored (LED Light Spectrum) light was used, which acted as treatment. A lighting structure on the roof of each rack was placed, to keep the PPFD constant at 100 $umolm^{-2}s^{-1}$. It was also attempted to keep the temperature and humidity at 18-20°C and 60%-80%, respectively. The lighting duration was 16 hrs/day with 8 hrs of darkness.

Plate 1: Preparation of germination pots for growing seedlings; where A. Cutting od rock wool for seed sowing, B. seeds of 3 different varieties, C. seed sowing on rockwool, D. Germination of seeds E. Different seedlings F. Second batch seedlings

Plate 2: Preparation of hydroponics nutrient solutions and seedlings transplanting; were A. Mixing of hydroponics nutrients a and b solution, B. Separations of seedlings from rockwool and set seedlings on net pots. C. Seedlings on net pot, D. setting of net pot over the hole in nutrient solutions

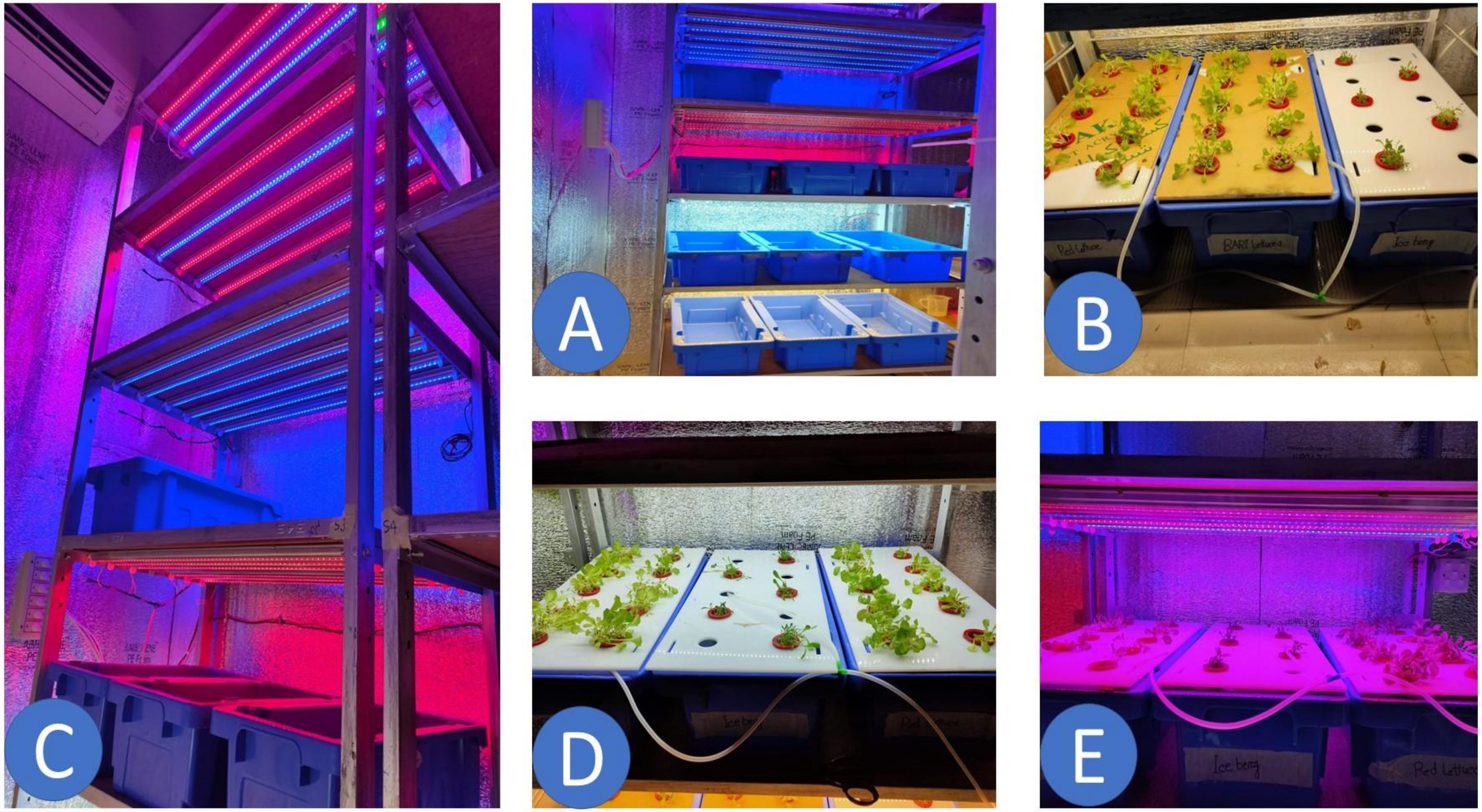

Plate 3: Seedlings transplanting; were A. Mixing of hydroponics nutrients a and b solution, B. Seedlings setup under Wr - Florescent Light (warn light), C. Light installation on Rack as treatments wise, D. Seedlings setup under White light (control), E. Seedlings setup under RB light

3.6.3 Transplanting of seedlings

When the seedlings' age was 20 days, they were ready to transplant (Plate 3). Individual cubes into several net pots were placed in such a way so that hydroponic solution could soak the rockwool, which helps supply nutrients to the plant. In this way, all the seedlings were prepared. On the other hand, after arranging the entire boxes in racks, all of them were filled with 25L of hydroponic solution with air stone for oxygen supply in each box by air pump. Each box contained five net pots, which represented five plants in the box. Transplanting was done by placing all the net pots over the solution. For air circulation in the entire room, we used a circular fan on one side of the rack in each light treatment area.

3.6.4 Harvesting

After 42 days of transplanting, all of the seedlings were ready to harvest (Plate 4). After harvesting, the fresh lettuce was kept in a cool place to avoid heat stress and collected the growth and yield data as well as prepared for qualitative testing.

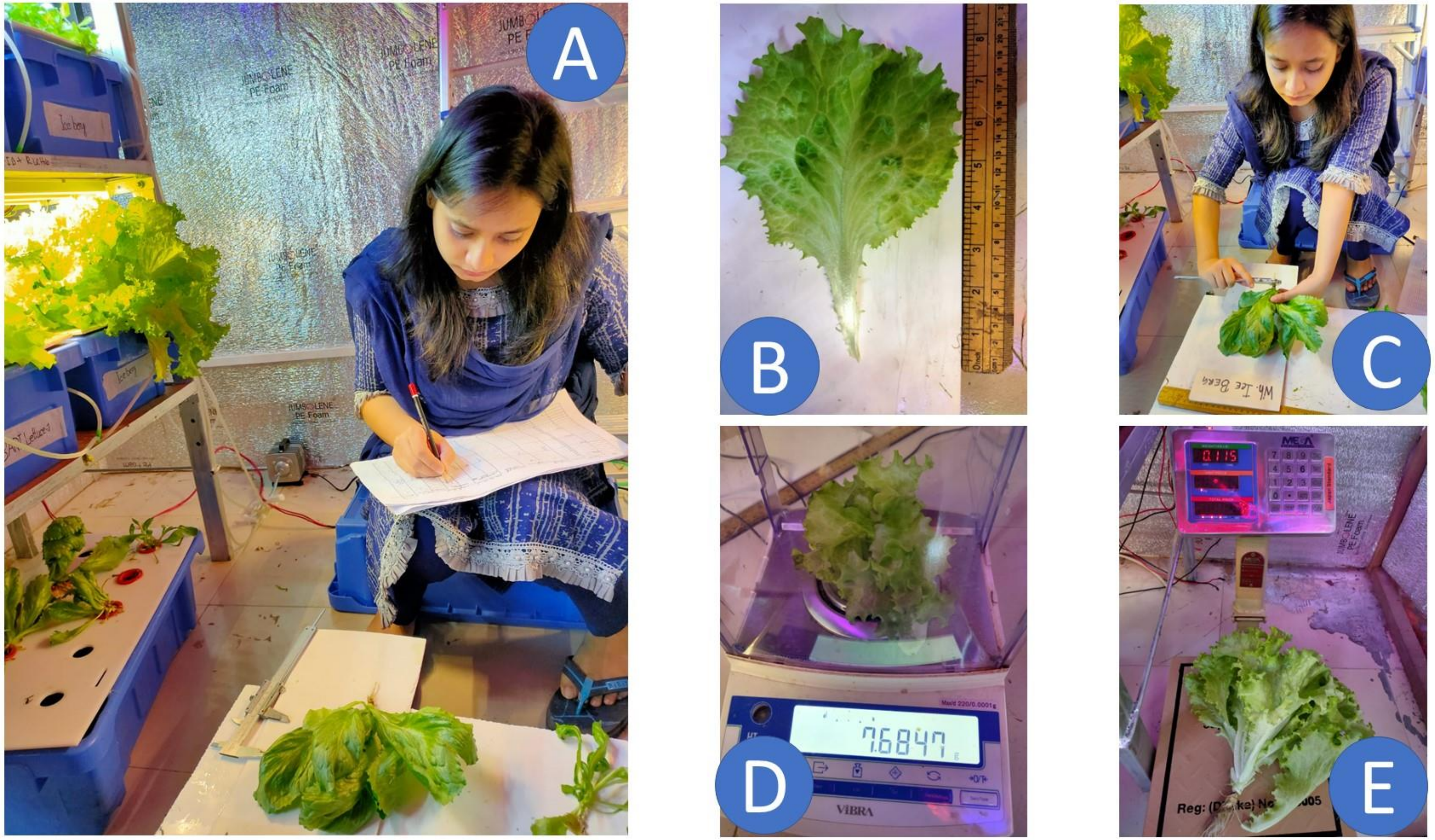

Plate 4: Harvesting and data collection; were A. Noted down the plant height data, B. Single plant leaf length measuring, C. Measuring stem diameter. D. Single leaf weight, E. Fresh weight per plant measuring

3.7 Recording of data

Then, every week, the physical data was taken for observation of growth physiological changes due to light spectrum on different varieties. The solution level was checked on a regular basis to see if the plant's roots got solution or not. The pH and EC of hydroponic solutions were checked on a regular basis with supervisor guidelines for further addition and balancing of pH and EC in nutrient solutions. Data was collected (Plate 5) on the following parameters:

A. **Growth parameters**
    1) Plant height (cm)
    2) Number of leaves plant^{-1}
    3) Leaf length (cm)
    4) Leaf breadth (cm)
B. **Yield contributing parameters and yield**
    1) Shoot diameter (cm)
    2) Tap Root length (cm)
    3) Fresh weight leaf^{-1} (g)
    4) Fresh weight plant^{-1} (g)
    5) Dry Weight (g)
    6) Yield pot^{-1} (kg)
    7) Yield (t ha^{-1})
C. **Quality parameters**
    1) Organoleptic Test
    2) Color Measurement
    3) SPAD value
    4) Vitamin-C content (mg. 100g^{-1} FW)
    5) Total Soluble Solid (°Brix)
    6) Anthocyanin (%)
    7) Antioxidant (mg/100gm)
    8) Economic Analysis

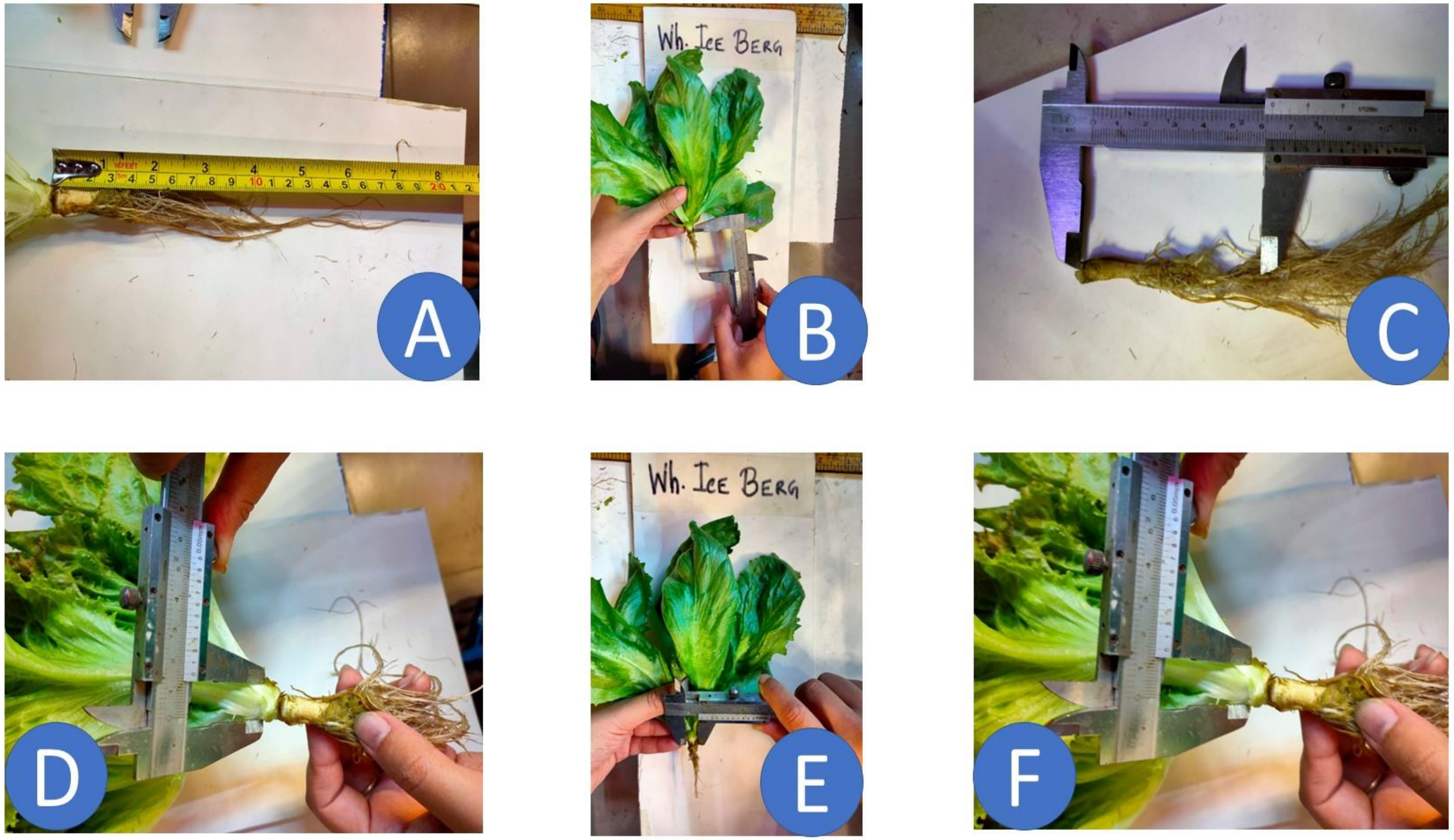

Plate 5: Harvesting and yield data collection; were A. Tap root length (BL1), B. Tap root length (IcB), C. Tap root length (RL). D. Stem diameter of BL1 variety, E. Shoot diameter of Ice burg, F. Shoot diameter of Red Lettuce (RL)

3.8 Procedure of recording data

A. Growth parameters

3.8.1 Plant height (cm)

The height of the plants was measured at each replication after 7 days of transplanting to a 42-DAT with a 7-days interval. The height was measured in centimeters (cm) from the ground level to the tip of the longest leaf, and the average height was calculated in centimeters.

3.8.2 Number of leaves plant^{-1}

The number of leaves per plant^{-1} was calculated from each replication, and the mean was recorded. The number of leaves plant^{-1} was measured from each replication from 7 to 42 DAT of every 7-days interval.

3.8.3 Leaf length (cm)

Leaf length was measured by using a meter scale. The measurement was taken from the base of the leaf to the tip of the petiole, and the average length of leaves was recorded from plants for each replication. Data was recorded from 7 to 42 DAT of every 7-days interval. The mean was expressed in centimeters (cm).

3.8.4 Leaf breadth (cm)

Leaf breadth was recorded as the average of five leaves selected at random from the plant of each replication from 7–42 DAT of every 7 days interval. Thus, the mean was recorded and expressed in centimeters (cm).

B. **Yield contributing parameters and yield**

3.8.5 Shoot diameter (cm)

The diameter of the shoot in centimeters (cm) was recorded from two plants of each treatment in each replication at the time of harvest (at 42 DAT) at the upper portion of the root of the plant where the leaves were attached to each other with slide calipers. Then the average value was used as a single treatment data point expressed in centimeters (cm).

3.8.6 Tap root length (cm)

Tap root length (cm) was recorded as the average of three plants selected at random from each replication at harvest. Thus, mean was recorded and expressed in centimeters (cm).

3.8.7 Fresh weight leaf $^{-1}$ (g)

At the time of harvest, three different single leaves of plant weight of each replication were taken, and the mean was recorded and expressed in gram (g).

3.8.8 Fresh weight plant^{-1} (g)

After removing roots and other stables from the plants, the whole plant weight of each replication was taken at harvest, and the mean was recorded and expressed in gram (g).

3.8.9 Dry Weight (g)

100 g fresh leaves from each replication from different treatments were chopped into small pieces and dried in the open air for one day, after which they were kept in the oven dryer for 48 hours. After getting the dry samples calculated as the previous value to present value, where means were recorded as dry weight (g).

3.8.10 Yield pot^{-1} (kg)

The yield of per pot was calculated by taking weight of each box in different treatment where mean value were recorded (Plate 8, 9)

3.8. 11 Yield t ha^{-1}

The yield of per ha was calculated by multiplying the yield per pot according to area measurement of different treatment where mean value were recorded.

3.8. 12 Organoleptic Test

A 25-member judge panel was formed. They evaluated the marketable qualities such as crispness, sweetness, bitterness, and appearance of lettuce leaves based on their acceptability. Scoring was done on the three categories, namely: Highly Acceptable (HA), Slightly Acceptable (SA) and Not Acceptable (NA) for all characters (Villared et al., 1979). Finally, acceptability score was done by using the following formula-

$$Highly\ Acceptable\ (HA) = \frac{Number\ of\ scroe\ x\ 100}{Total\ number\ of\ Judges} x7$$

$$Slightly\ Acceptable\ (SA) = \frac{Number\ of\ scroe\ x\ 100}{Total\ number\ of\ Judges} x5$$

$$Not\ Acceptable\ (NA) = \frac{Number\ of\ scroe\ x\ 100}{Total\ number\ of\ Judges} x2$$

Finally, the lettuce leaves were sorted into three categories on the basis of score.

3.8.13 Color Measurement

Color was measured with a colorimeter (iWave, WF28, China) using the CIE Lab L*, a*, b*, and c*color scale. The L* value is the lightness parameter indicating the degree of lightness of the sample; it varies from 0 = black (dark) to 100 = white (light). The value a*, which is the chromatic redness parameter whose value means tending to red color when positive (+a*) and green color when negative (-a*). The b* is the yellowness chromatic parameter corresponding to yellow color when it is positive (b*) and blue color when it is negative (-b*). Chroma = was calculated and higher numbers of chromaticity indicate a more vivid color, whereas lower numbers correspond to dull colors. Color measurement was done just after the harvesting of lettuce.

3.8.14 SPAD Value

The SPAD value was measured by a Chlorophyll Meter (SPAD-502 Plus KONICA MINOLTA, Japan) at room temperature. Firstly, 3 leaves of each plant considered in all replication data were collected to determine the chlorophyll by SPAD value.

3.8.15 Vitamin C content (mg. 100 g^{-1} FW)

Ascorbic acid was quantitatively determined according to the 2,6 dichlorophenolindophenol-dye method as described by Jones and Hughes (1983) with slight modifications. The ascorbic acid in 10 g of fresh sample was extracted by grinding with a small amount of acid-washed quartz sand and 3% meta-phosphoric acid (v/v). The extract volume was made up to 100 ml, mixed and centrifuged at 3000 g for 15 min at room temperature (Plate 7). 10 ml were titrated against standard 2, 6-dichlorophenolindophenol dye, which was already standardized against standard ascorbic acid. Results were expressed in mg.100 g^{-1} FW.

3.8.16 Total Soluble Solid (°Brix)

TSS was measured by a refractometer (Hanna Instruments, HI96801, Romania) at room temperature (Plate 6). To begin, three replicated leaves from each plant were blended with a mortar and pestle to extract juice, which was then calculated and expressed as a percentage.

Plate 6: ^{0}Brix measurement; were A. 9 treatment combination samples B. Operating refractometer for measuring %brix, C. Reading of refractometer %brix

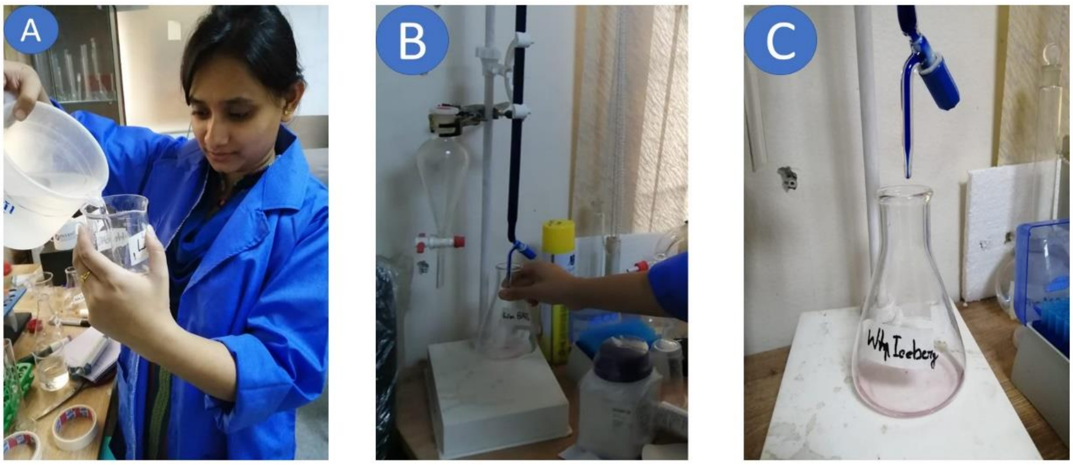

Plate 7: Vitamin C Content measurement by titration; were A. Diluting samples extract, B. Preparation of methyl blue in burette, C. Pick color observation for finishing the titration

3.8.17 Antioxidant activity (%)

Methanol extracts of freeze-dried leaves were prepared for the determination of antioxidant activity. Weighed leaf samples (5 g) were placed in a glass beaker and homogenized with 50 mL of methanol at 24◦C overnight (Plate 8). The homogenate was filtered and then centrifuged at 6000 rpm for 15 min. The free radical scavenging activity of the samples was determined using the 2,2,-diphenyl-2-picrylhydrazyl (DPPH) method (Turkmen *et al*., 2005). An aliquot of 2 ml of 0.15 mM DPPH radical in methanol was added to a test tube with 1 ml of the sample extract. The reaction mixture was vortex mixed for 30 s and left to stand at room temperature in the dark for 20 min. The absorbance was measured at 517nm using a visible spectrophotometer (Hanna Instruments, Iris HI801, Romania) (plate 12). The antioxidant activity was calculated using the following equation:

Antioxidant activity (%) = 1 - A Sample (517 nm)/A Control (517 nm) ×100

3.8.18 Determination of anthocyanin (mg·100 g^{-1} FW)

The pigment (anthocyanin, at 500 and 900nm) of the leaves was investigated with a visible spectrophotometer (Hanna Instruments, Iris HI801, Romania). Three equivalent aged leaves from each tunnel were collected early in the morning. Each sample was extracted with 15 ml of metahanol: HCl (99:1) and placed in a vial. Then the procedure was followed according to Tsormpatsidis *et al*. (2010) and then the results were expressed as mg $100g^{-1}$ fresh weight (FW) (Plate 9). The absorbance measurement was done within 20-50 min of preparation.
The anthocyanin pigment concentration expressed as cyaniding-3-glucoside equivalent, as follows:

Anthocyanin pigment (cyaniding-3-glucoside equivalents, mg·100 g^{-1} FW)

$$= \frac{\text{A x MW x DF x 1000}}{\varepsilon \text{ x 1}}$$

Where, A = (A500nm- A900nm) pH 1.0 – (A500nm – A900nm) pH 4.5; MW (molecular weight) = 449.2 g.mol-1for cyaniding-3-glucoside; DF = dilution factor; 1 = path length in cm; e = 26, 900 molar extinction coefficients, in L × mol 1× cm-1, for cyaniding-3-glucoside and 1000 = factor for conversion from g to mg.

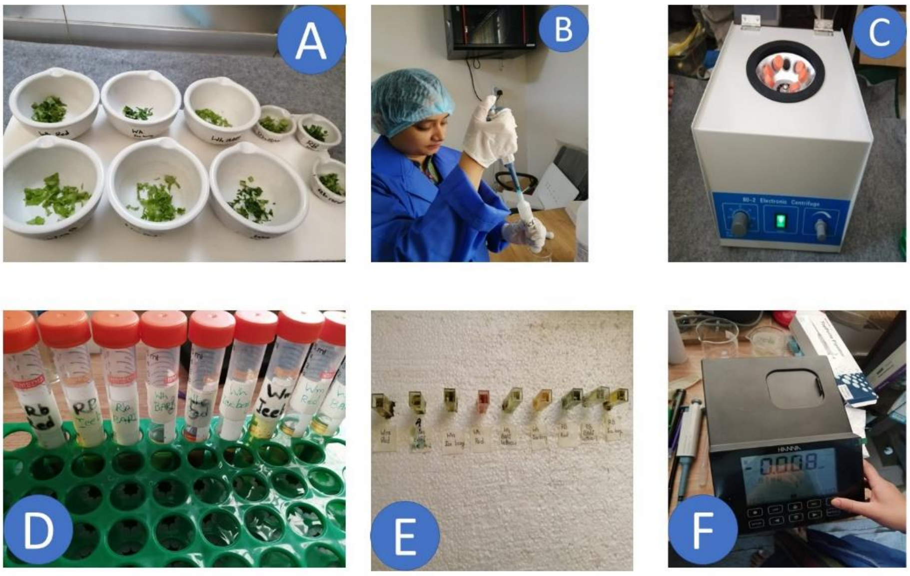

Plate 8: Preparation for anthocyanin measurement; were A. Sample collection and weighting for smashing, B. HCL measurement for dilute with sample, C. Centrifuge the sample with falcon tube, D. Collection of leaves extract in falcon tube, E. collecting the plant extract solution in cuvette, F. Operating spectrophotometer

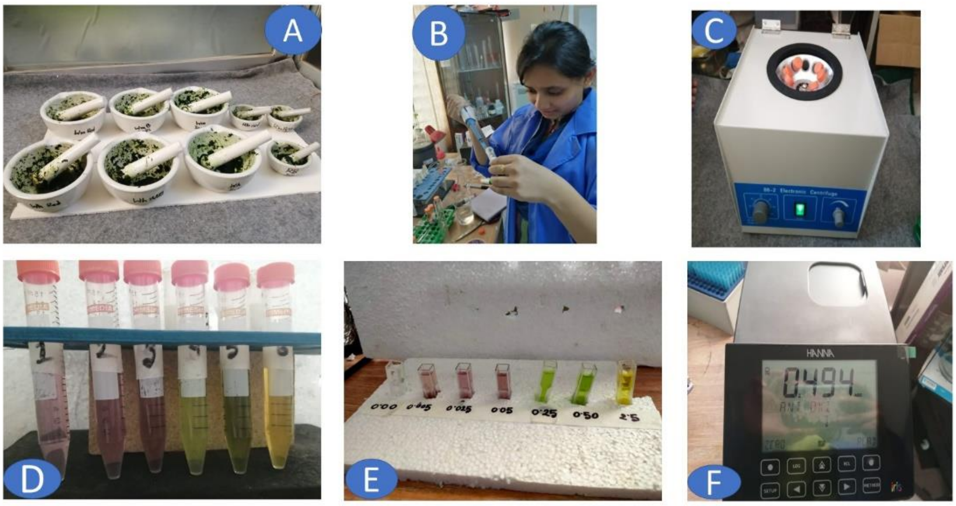

Plate 9: Preparation for antioxidant measurement; were A. Sample collection and weighting for smashing, B. Colleting plant samples from falcon tube, C. Centrifuge the sample for extract, D. Different sample extract E. Samples in different concentration, F. Spectrophotometer reading for antioxidant measurement

3.8.19 Economic analysis

The cost of production was calculated to find the most economical combination of variety and different color shade nets. All input costs, like the cost of land lease and interest on running capital, were computed in the calculation. The interest was calculated at a simple rate of 13%. The market price of lettuce was used for estimating the return.

The benefit cost ratio (BCR) was calculated as follows: The benefit cost ratio (BCR) was calculated by the following formula.

$$\text{Benefit Cost Ratio} = \frac{\text{Gross Return (Tk/ha)}}{\text{Total Cost of Production}}$$

3.9 Statistical analysis

The collected data on various parameters under study were statistically analyzed using Statistic10 computer package programme. The means for all the treatments were calculated and analysis of variance for all the characters was performed by the F-variance test (Gomez and Gomez, 1984). Significance of difference between means was evaluated by Least Significance Difference (LSD) and the probability level 5% and 1% for the interpretation of results.

CHAPTER IV

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) of the data on different characters have been presented in Appendix IV-XI. The results have been presented and discussed with the interpretations are given under the following headings:

A. Growth parameters

4.1 Plant Height (cm)

4.1.1 Effect of light spectrum on plant height

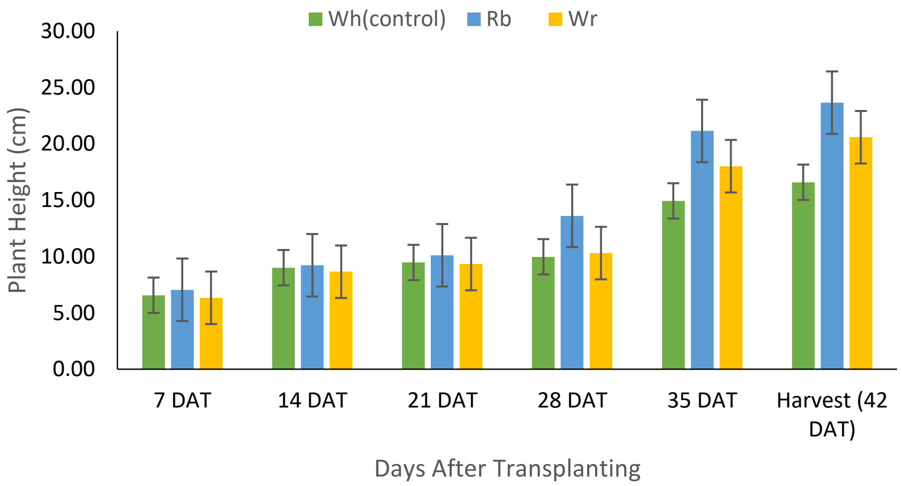

Figure 1: Plant height of lettuce as influenced by different LED light spectrum at different days after transplanting

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1

Due to various light spectrum effects, plant height of lettuce exhibited statistically significant variations at 7, 14, 21, 28, 35 DAT and harvest time (Figure 1, Appendix IV). The highest plant heights of 7.05 cm, 9.22 cm, 10.11 cm, 13.61 cm, 21.14 cm, and 23.66 cm were observed at 7, 14, 21, 28, 35 DAT and harvest time, respectively for Rb treatment. While the lowest plant height of 6.33 cm, 8.65 cm, and 9.33 cm was noted at 7, 14, and 21 DAT for treatment with Wr. At 28, 35 DAT and harvest time, the shortest plant was 9.98 cm, 14.94 cm and 16.59 cm, respectively, for control treatment (WhC).

4.1.2 Effect of variety on plant height

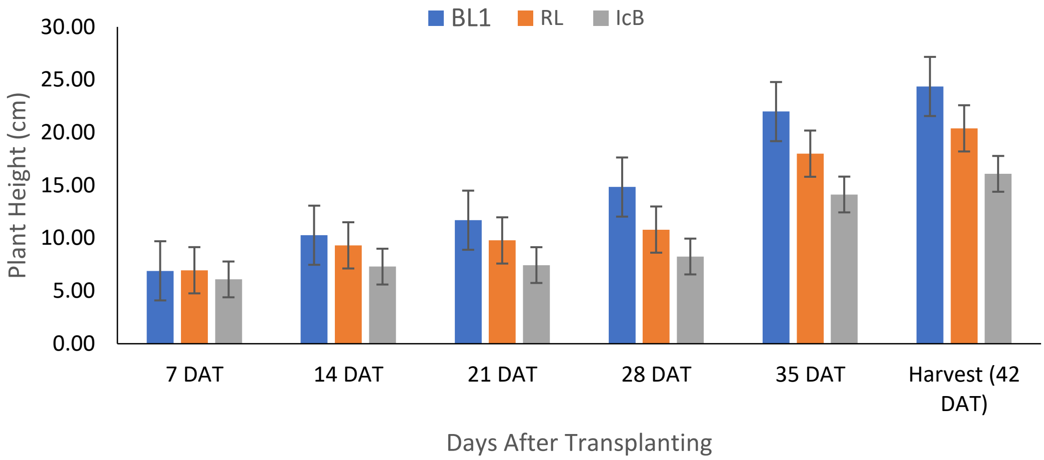

Figure 2: Plant height of lettuce as influenced by different varieties at different days after transplanting

Here, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

Different varieties of lettuce exhibited significant variation in plant height at 7, 14, 21, 28, 35 DAT and harvest time (Figure 2, Appendix IV). Only Red lettuce (RL) at 7 DAT showed a maximum plant height of 6.96 cm, while at 14, 21, 28, 35 DAT and harvest time, maximum plant heights of 10.27 cm, 11.69 cm, 14.83 cm, 21.98 cm and 24.36 cm were observed respectively for (BL1) BARI lettuce 1. On the other hand, the smallest plant heights of 6.09 cm, 7.30 cm, 7.44 cm, 8.26 cm, 14.13 cm, and 16.08 cm were recorded at 7, 14, 21, 28, 35 DAT and harvest time for the (IcB) Iceberg variety of lettuce.

4.1.3 Combined effect of different LED light spectrum and variety on plant height

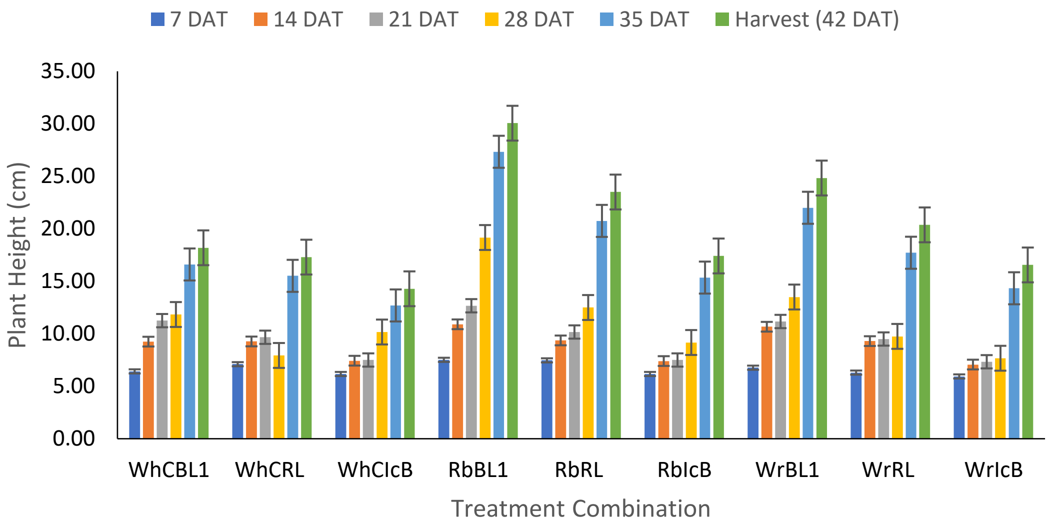

Figure 3: Plant height of lettuce as influenced by different LED light spectrum and varieties at different days after transplanting

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

The interaction effect of light spectrum and variety of lettuce exhibited significant variation for plant height at 7, 14, 21, 28, 35DAT and harvest time (Figure 3, Appendix IV). The tallest plants, 7.52 cm, 10.90 cm, 12.67 cm, 19.17 cm, 27.33 cm, and 30.06 cm, were noted at 7, 14, 21, 28, 35 DAT and harvest time, respectively for the RbBL1 treatment combination. On the contrary, the (IcB) Iceberg variety of lettuce presented the smallest plants at 5.93 cm, 7.07 cm, 7.33 cm, and 7.67 cm at 7, 14, 21, and 28 DAT, respectively, in Wr spectrum treatment. Moreover, at 35 DAT and harvest time, the lowest heights of 12.70 cm and 14.28 cm, respectively, were observed for (IcB) Ice Berg in control treatment (WhC) (Plate 7,8). Huang *et al*. (2021) conducted a study aimed to evaluate the effects of alternating red (660 nm) and blue (460 nm) light on the growth and nutritional quality of two-leaf-color Lettuce (*Brassica campestris* L. ssp. *chinensis* var. communis). The present study indicated that the biomass of green-leaf Lettuce was much higher than that of red-leaf Lettuce , but the nutritional quality of green-leaf Lettuce was lower than that of red-leaf Lettuce , and seemingly indicating that the regulation of metabolism for Lettuce was species specific under light exposure.

4.2 Number of leaves plant^{-1}

4.2.1 Effect of light spectrum on number of leaves plant^{-1}

Table 1: Number of leaves plant^{-1} of lettuce as influenced by Different LED light spectrum at different days after transplanting

Treatment	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	Harvest (42 DAT)
WhC	4.44 b	5.44 a	8.55 b	10.22 b	12.22 b	14.33 a
Rb	5.33 a	6.00 a	9.44 a	11.22 a	13.88 a	15.88 a
Wr	4.66 ab	4.77 b	8.88 b	10.33 b	12.55 b	14.55 a
CV (%)	13.99	11.04	5.83	6.26	12.64	11.43
LSD	0.67	0.59	0.52	0.66	1.62	1.70

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

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1

Number of leaves plant^{-1} of lettuce exhibited significant variations due to various light spectrum effects at 7, 14, 21, 28, 35 DAT and harvest time (Table 1, Appendix IV). 5.33, 6.00, 9.44, 11.22, 13.88, and 15.88 were counted for Rb light at 7, 14, 21, 28, and 35 DAT and harvest time, respectively. On the contrary, at 14 DAT (Wr) treatment showed the lowest number (4.77) of lettuce leaves. While, at 7, 21, 28, 35 DAT and harvest time, the lowest number of lettuce leaves was 4.44, 8.55, 10.22, 12.22, and 14.33, respectively in the control treatment (WhC).

4.2.2 Effect of varieties on number of leaves plant^{-1}

Table 2: Number of leaves plant^{-1} of lettuce as influenced by Different lettuce varieties at different days after transplanting

Treatment	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	Harvest (42 DAT)
BL1	5.22 a	5.66 a	10.11 a	11.88 a	13.88 a	15.88 a
RL	4.88 ab	5.44 a	9.55 b	11.00 b	14.22 a	16.33 a
IcB	4.33 b	5.11 a	7.22 c	8.88 c	10.55 b	12.55 b
CV (%)	13.99	11.04	5.83	6.26	12.64	11.43
LSD	0.67	0.59	0.52	0.66	1.62	1.70

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

Here, BL1- BARI Lettuce-1, RL- Red Lettuce IcB- Ice Berg Lettuce]

At 7, 14, 21, 28, 35 DAT and harvest time, there was statistically significant variation in the number of leaves due to the use of different varieties (Table 1, Appendix IV). At 7, 14, 21, and 28 DAT, the highest number of leaves of 5.22, 5.66, 10.11, and 11.88 were observed for (BL1) BARI lettuce 1. While at 35 DAT and harvest time, the maximum number of leaves in an individual lettuce plant were 14.22 and 16.33, respectively, from (RL) red lettuce. On the other hand, the minimum number of leaves 4.33.11, 7.22, 8.88, 10.55, and 12.55 were observed for the (IcB) Iceberg lettuce variety at 7, 14, 21, 28, 35 DAT and harvest time, respectively.

4.2.3 Combined effect of different LED light spectrum and variety on number of leaves plant^{-1}

Table 3: Number of leaves plant^{-1} of lettuce as influenced by different LED light spectrum and lettuce varieties at different days after transplanting

Treatment	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	Harvest (42 DAT)
WhCBL1	4.66 bc	5.00 bc	9.66 b	11.33 b	13.33 bc	15.33 bcd
WhCRL	4.00 c	5.33 b	9.33 b	10.66 b	13.33 bc	15.66 abc
WhCIcB	4.66 bc	6.00 ab	6.66 d	8.66 c	10.00 d	12.00 e
RbBL1	6.00 a	6.66 a	11.00 a	13.00 a	14.33 ab	16.33 ab
RbRL	5.66 ab	6.00 ab	9.66 b	11.66 b	16.33 a	18.33 a
RbIcB	4.33 c	5.33 b	7.66 c	9.00 c	11.00 cd	13.00 cde
WrBL1	5.00 abc	5.33 b	9.66 b	11.33 b	14.00 ab	16.00 ab
WrRL	5.00 abc	5.00 bc	9.66 b	10.66 b	13.00 bc	15.00 bcd
WrIcB	4.00 c	4.00 c	7.33 cd	9.00 c	10.66 cd	12.66 de
CV (%)	13.99	11.04	5.83	6.26	12.64	11.43
LSD	1.16	1.03	0.90	1.14	2.81	2.95

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

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

Due to the interaction effect of light spectrum and lettuce variety, lettuce showed significant variation in leaf number at 7, 14, 21, 28, 35 DAT and harvest time (Table 3, Appendix IV). Number of leaves were observed to be highest (6.00, 6.66, 11.00, and 13.00) in (RbBL1) treatment combination at 7, 14, 21, and 28 DAT, respectively for (BL1) BARI Lettuce-1. Moreover, (RbRL) Red Lettuce exhibits a maximum number of leaves of 16.33, 18.33 at 35 DAT, at harvest, respectively. On the contrary, the number of leaves was found to be lowest for both RbIcB (4.33) and RbIcB (4.00). Rb light treatment combination with Ice Berg showed, respectively, at 7 DAT. Furthermore, at 21, 28, 35 DAT and harvest time, the lowest number of leaves, 6.66, 8.66, 10.00, and 12.00, were noted for Ice Berg in control treatment (WhCIcB).

4.3 Leaf Length (cm)

4.3.1 Effect on different LED light spectrum on leaf length

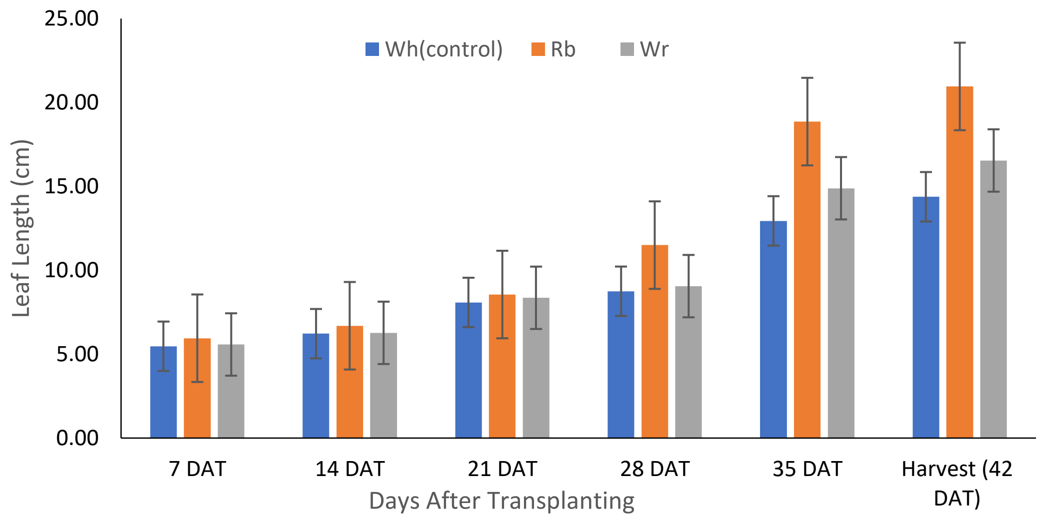

Figure 4: Leaf length of lettuce as influenced by different LED light spectrum at different days after transplanting

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1

Considerable variation was observed for the leaf length of lettuce due to different light spectrums at 7, 14, 21, 28, 35 DAT and harvest time (Figure 4, Appendix V). The highest leaf lengths were found at 5.95 cm, 6.69 cm, 8.56 cm, 11.50 cm, 18.86 cm, and 20.96 cm, in (Rb) light treatment at 7, 14, 21, 28, 35 DAT and at harvest time, respectively. While the (WhC) control treatment exhibited lower leaf length, 5.47 cm, 6.22 cm, 8.08 cm, 8.75 cm, 12.94 cm, and 14.38 cm at 7, 14, 21, 28, and 35 DAT and at harvest time, respectively.

4.3.2 Effect on different varieties on leaf length

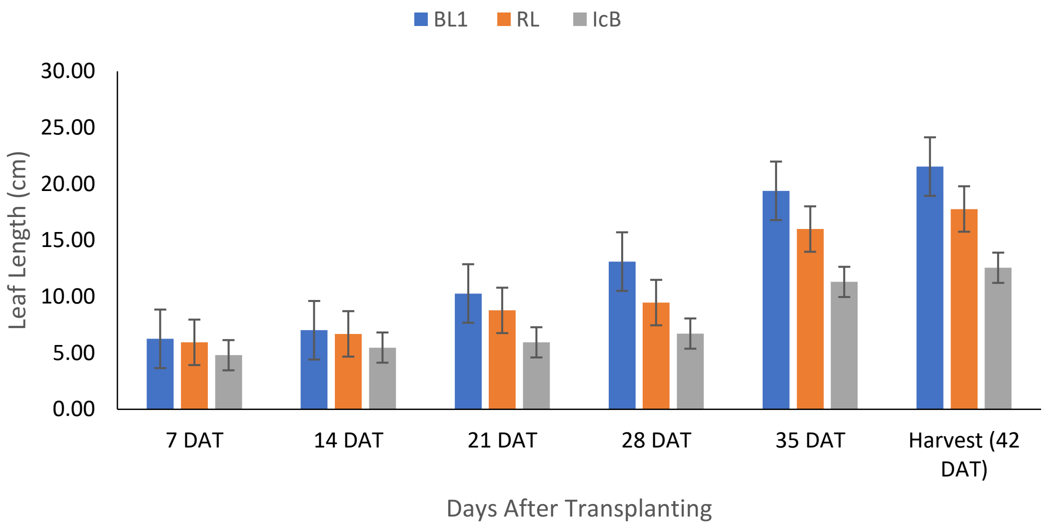

Figure 5: Leaf length of lettuce as influenced by different varieties at different days after transplanting

Here, BL1- BARI Lettuce-1, RL- Red Lettuce IcB- Ice Berg Lettuce

The length of lettuce leaves exhibited statistically significant variation at 7, 14, 21, 28, 35 DAT and harvest time for different varieties of lettuce (Figure 5, Appendix V). BARI lettuce1 (BL1) presented the largest leaf lengths of 6.26 cm, 7.02 cm, 10.28 cm, 13.11 cm, 19.39 cm, and 21.54 cm at 7, 14, 21, 28, and 35 DAT and harvest time, respectively. On the contrary, the minimum length of a leaf was 4.80 cm, 5.48 cm, 5.94 cm, 6.72 cm, 11.31 cm, and 12.56 cm, recorded from the (IcB) Iceberg variety of lettuce at 7, 14, 21, 28, and 35 DAT and harvest time, respectively.

4.3.3 Combined effect of different varieties and LED light spectrum on leaf length

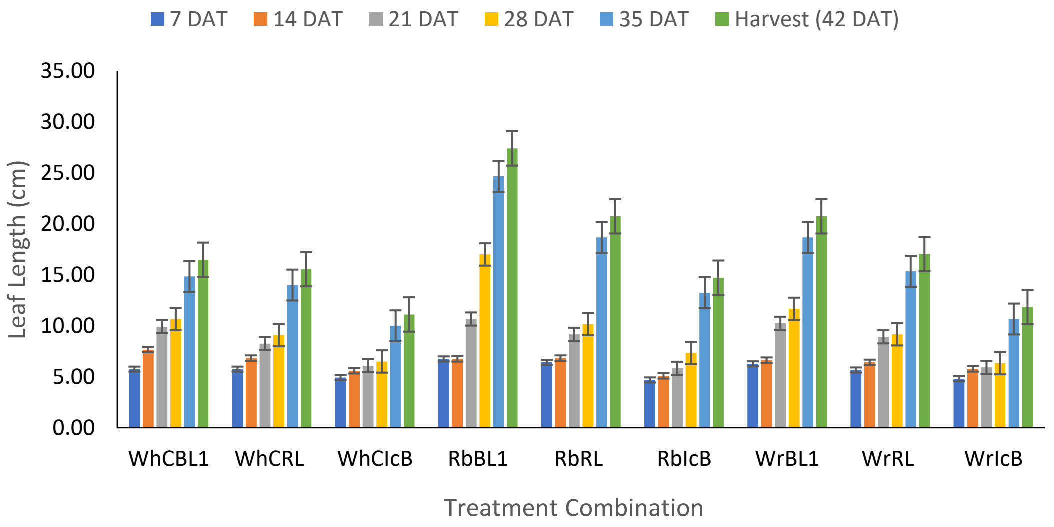

Figure 6: Leaf length of lettuce as influenced by different LED light spectrum and varieties at different days after transplanting

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

Because of the interaction of the light spectrum with lettuce varieties, significant variations in lettuce leaf length were observed at 7, 14, 21, 28, 35, and harvest time (Figure 6, Appendix V). The longest lengths were found at 6.75 cm, 10.67 cm, 17.00 cm, 24.67 cm, and 27.41 cm observed for BL1 under (Rb) light treatment at 7, 21, 28, and 35 DAT and harvest time, respectively, whereas the shortest lengths of (IcB) iceberg under (Rb) light were 4.69 cm, 5.08 cm, and 5.83 cm at 7, 14, and 21DAT, respectively. While, at 28 DAT, the smallest length (6.33 cm) was recorded for Iceberg under Wr light. Moreover, Iceberg exhibited a minimum leaf length of 10.00 cm and 11.11 cm at 35 DAT and harvest time, respectively, under (WhC) white light.

4.4 Leaf Breadth (cm)

4.4.1 Effect on different LED light spectrum on leaf breadth

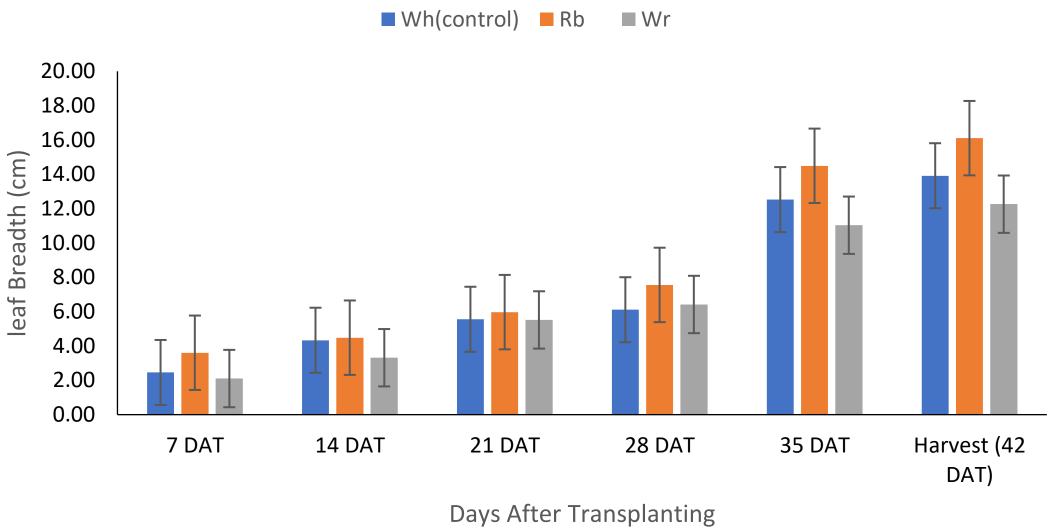

Figure 7: Leaf breadth of lettuce as influenced by different LED light spectrum at different days after transplanting

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1

Leaf breath exhibited significant variation due to different light spectrum treatments at 7, 14, 21, 28, 35 DAT and harvest time (Figure 7, Appendix V). (Rb) light treatment presented the longest breadth of leaf (3.61 cm, 4.48 cm, 5.97 cm, 7.56 cm, 14.49 cm, and 16.11 cm) at 7, 14, 21, 28, 35 DAT and harvest time, respectively. On the other hand, the lowest breath of a lettuce leaf was 2.10 cm, 3.32 cm, 5.52 cm, 11.03 cm, and 12.26 cm were recorded at 7, 14, 21, 35 DAT and harvest time, respectively, and only at 28 DAT the smallest breath was 6.11 cm due to (WhC) control treatment.

4.4.2 Effect on different varieties on leaf breadth

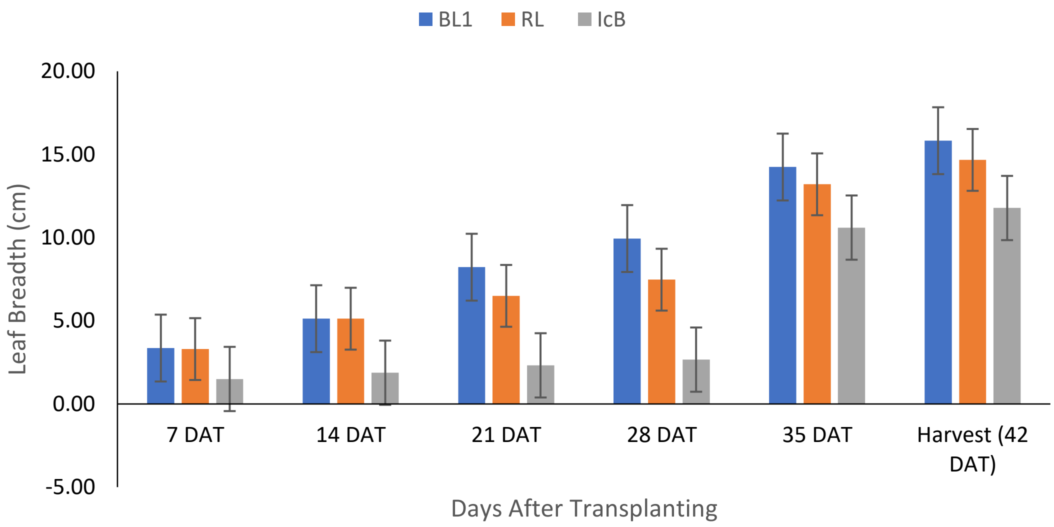

Figure 8: Leaf breadth of lettuce as influenced by different varieties at different days after transplanting

Here, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

At 7, 14, 21, 28, 35 DAT and harvest time, leaf breadth showed statistically significant variations due to different varieties of lettuce (Figure 8, Appendix V). (BL1) BARI lettuce 1 expressed the largest breadth at 3.36 cm, 5.13 cm, 8.22 cm, 9.94 cm, 14.24 cm, and 15.83 cm for lettuce leaves at all observed DAT, as well as (RL) Red Lettuce, which also showed maximum breath at 14 DAT. On the contrary, the lowest length of 1.50 cm, 1.88 cm, 2.32 cm, 2.67 cm, 10.60 cm, and 11.78 cm of lettuce leaf were recorded for Ice Berg at 7, 14, 21, 28, 35 DAT and harvest time, respectively.

4.4.3 Combined effect of different varieties and LED light spectrum on leaf breadth

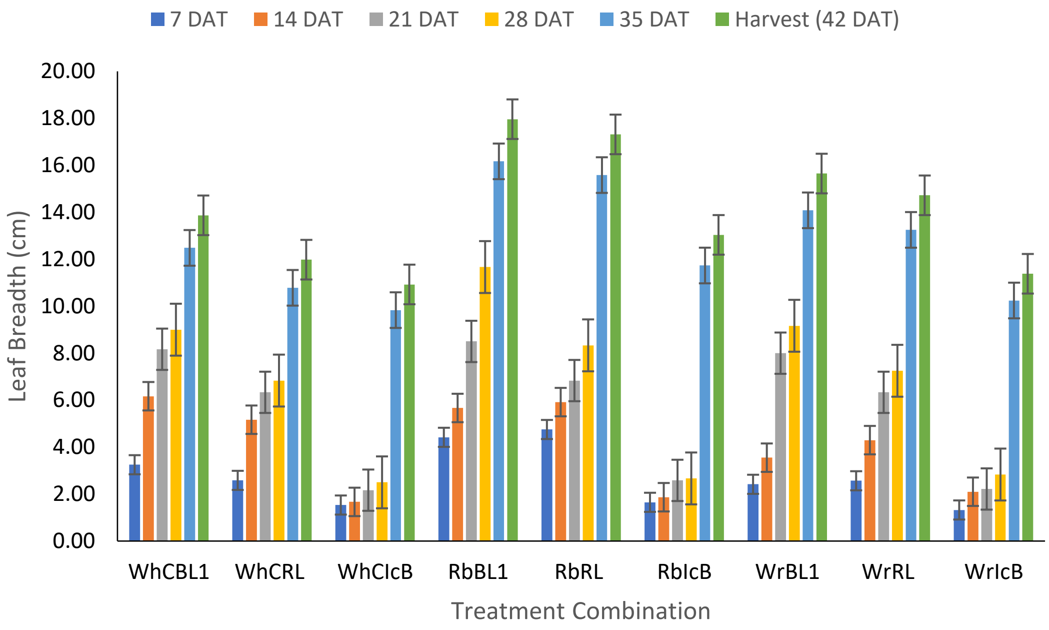

Figure 9: Leaf breadth of lettuce as influenced by different LED light spectrum with varieties at different days after transplanting

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

The combined effect of light spectrum and lettuce varieties exhibited significant variation for leaf breath at 7, 14, 21, 28, 35 DAT and harvest time (Figure 9, Appendix V). At 7 DAT, (RL) Red Lettuce presented a maximum breadth of 4.75 cm under Rb light, while (BL1) showed the largest breadth of 6.17 cm at 14 DAT under (WhC) control treatment. Besides that, BL1 exhibited the highest breadth of 8.50 cm, 11.67 cm, 16.17 cm, and 17.96 cm under Rb light conditions at 21, 28, and 35 DAT and harvest time, respectively. On the other hand, (IcB) iceberg at 7 DAT showed the smallest breadth of 1.32 cm under Wr treatment, while the shortest breadth of 1.67 cm, 2.17 cm, 2.50 cm, 9.83 cm, and 10.93 cm for other observed DAT was recorded under (WhC) light treatment.

B. Yield contributing parameters and yield of lettuce

4.5 Shoot Diameter (cm)

4.5.1 Effect of light spectrum on shoot diameter

The diameter of the shoot of lettuce exhibited significant variation due to the use of different light spectrum treatments (Table 4, Appendix VII). (Rb) light presented the best result for the length of shoot diameter, which was 1.32 cm. In contrast, the lowest result of 0.99 cm was observed under control treatment (WhC).

4.5.2 Effect of variety on shoot diameter

Different varieties of lettuce express remarkable variation in shoot diameter (Table 4, Appendix VII). Red lettuce had the largest lettuce shoot diameter of 1.29 cm. Iceberg was observed to have the lowest shoot diameter, which was 1.06 cm, respectively.

4.5.3 Combined effect of different LED light spectrum and variety on shoot diameter

The interaction effects of light spectrum and variety caused significant variation in shoot diameter (Table 4, Appendix VI). Under the Rb light treatment, shoot diameter was the highest, which was 1.30 cm and 1.50 cm for (BL1) and (RL) lettuce, respectively. On the contrary, Iceberg exhibited the lowest shoot diameter (0.86 cm) control treatment (WhCIcB).

4.6 Tap root length (cm)

4.6.1 Effect of light spectrum on tap root length

The tap root length of lettuce exhibited significant variation due to the use of different light spectrum treatments (Table 4, Appendix VII). (Rb) light presented the highest result for the length of tap root, which was 8.64 cm. In contrast, the lowest result of 8.29 cm was observed under control treatment (WhC).

4.6.2 Effect of variety on tap root length

Tap root length varied significantly between lettuce varieties (Table 4, Appendix VII). The tap root of BARI lettuce was observed at its highest (9.22 cm). Iceberg was observed to have the lowest tap root length, which was 8.08 cm.

4.6.3 Combined effect of different LED light spectrum and variety on tap root length

The length of the tap root varied significantly due to the interaction effects of light spectrum and variety (Table 4, Appendix VII). Under the warm light treatment, tap root length was the highest, which was 9.66 cm for BARI lettuce-1. On the contrary, RL-Red Lettuce exhibited the lowest tap root length (7.23 cm) under control treatment.

Table 4. Tap root length and shoot diameter of lettuce influenced by LED light spectrum

Treatment	Tap Root Length (cm)	Shoot Diameter (cm)
Wh(control)	8.29 a	0.99 b
Rb	8.64 a	1.32 a
Wr	8.47 a	1.16 ab
CV (%)	12.00	15.77
LSD	1.01	0.18

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

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1

Table 5. Tap root length and shoot diameter of lettuce influenced by varieties

Treatment	Tap Root Length (cm)	Shoot Diameter (cm)
BL1	9.22 a	1.12 ab
RL	8.11 b	1.29 a
IcB	8.08 b	1.06 b
CV (%)	12.00	15.77
LSD	1.01	0.18

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

Here, BL1- BARI Lettuce-1; RL- Red Lettuce; IcB- Ice Berg Lettuce

Table 6. Tap root length and shoot diameter of lettuce influenced by LED light spectrum and varieties

Treatment	Tap Root Length (cm)	Shoot Diameter (cm)
WhCBL1	9.58 a	0.93 cd
WhCRL	7.23 b	1.16 bcd
WhCIcB	8.06 ab	0.86 d
RbBL1	9.66 a	1.30 ab
RbRL	8.45 ab	1.50 a
RbIcB	7.8 b	1.16 bcd
WrBL1	8.4 ab	1.13 bcd
WrRL	8.63 ab	1.20 abc
WrIcB	8.36 ab	1.13 bcd
CV (%)	12.00	15.77
LSD	1.75	0.31

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

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

4.7 Fresh weight (g) leaf^{-1}

4.7.1 Effect of light spectrum on fresh weight (g) leaf^{-1}

Considerable variations were noticed in the fresh weight of a single leaf. (Rb) light treatment was observed to give a maximum fresh weight of leaf^{-1} (10.05 g). In contrast, a minimum fresh weight of 8.87 g per leaf was noticed for (Table 7, Appendix VII) control treatment (WhC).

4.7.2 Effect of variety on fresh weight (g) leaf^{-1}

Due to different lettuce varieties, the fresh weight per leaf showed remarkable variations. The (BL1) variety produced the highest fresh weight per leaf of 9.84 g, while (RL) produced the lowest fresh weight per leaf of 9.14 g. (Table 8, Appendix VII)

4.7.3 Combined effect of different LED light spectrum and variety on fresh weight (g) leaf^{-1}

Due to the interaction between the light spectrum and lettuce varieties, fresh weight per leaf exhibited significant variations (Table 9, Appendix VII). BARI Lettuce 1 exhibited the highest fresh weight per leaf (10.53 g) due to the (RbBL1) treatment combination. while, due to the control treatment with Red Lettuce (WhCRL), showed the lowest fresh weight per leaf, which is 7.8 g.

4.8 Fresh weight plant^{-1} (kg)

4.8.1 Effect of light spectrum on fresh weight plant^{-1} (kg)

Remarkable variation was observed for the yield of fresh weight plant^{-1} for (Rb) and (Wr) light treatments. The maximum result for fresh weight plant^{-1} was 0.14 kg plant^{-1}. while a minimum of 0.12 kg plant^{-1} was recorded in the control treatment (WhC) (Table 7, Appendix VII). Yan *et al*. (2020) reported that red plus blue light-emitting diodes (LEDs) are commonly applied in plant factories with artificial lighting due to photosynthetic pigments, the results indicated that the red plus blue LEDs increased leaf fresh and dry weights of green leaf lettuce by 28% and 34%, respectively.

4.8.2 Effect of variety on fresh weight plant^{-1} (kg)

Due to different lettuce varieties, the fresh weight per plant exhibited considerable variations. (Table 8, Appendix VII). The Iceberg variety gave the maximum fresh weight per plant, which is 0.14 kg, while both BARI lettuce 1 and red lettuce expressed a minimum result for fresh weight per plant, which is 0.13 kg.

4.8.3 Combined effect of different LED light spectrum and variety on fresh weight plant^{-1} (kg

Significant variations were recorded for fresh weight plant^{-1} due to the interaction between the light spectrum and lettuce varieties (Table 9, Appendix VI). Iceberg and the combination of Rb light treatment exhibited the highest fresh weight plant^{-1} RbIcB (0.16 kg). while, in the control treatment, (WhCRL) Red Lettuce showed the lowest fresh weight plant^{-1}, which was (0.118 kg).

4.9 Dry weight (g)

4.9.1 Effect of light spectrum on dry weight (g)

Dry weight exhibited remarkable variation due to different levels of the light spectrum. Rb light treatment produced the highest dry weight ever noted (17.24 g) (Table 7, Appendix VII). whereas the (WhC) control treatment expressed the minimum fresh weight, which was 14.48g.

4.9.2 Effect of variety on dry weight (g)

Different lettuce varieties showed significant variations in dry weight. A maximum dry weight of 16.75 g was recorded for the Iceberg variety (Table 8, Appendix VII). While, Red Lettuce gave the minimum result for dry weight, which was 15.24 g.

4.9.3 Combined effect of different LEDs light and variety on dry weight (g)

Due to the combination of light spectrum and lettuce varieties, dry weight exhibited significant variations (Table 9, Appendix VII). The highest dry weight (18.5 g) was recorded for Iceberg combined with Rb light treatment (RbIcB). while, due to the light control treatment, the lowest dry weight (13.57 g) was recorded for the (WhCRL) treatment combination.

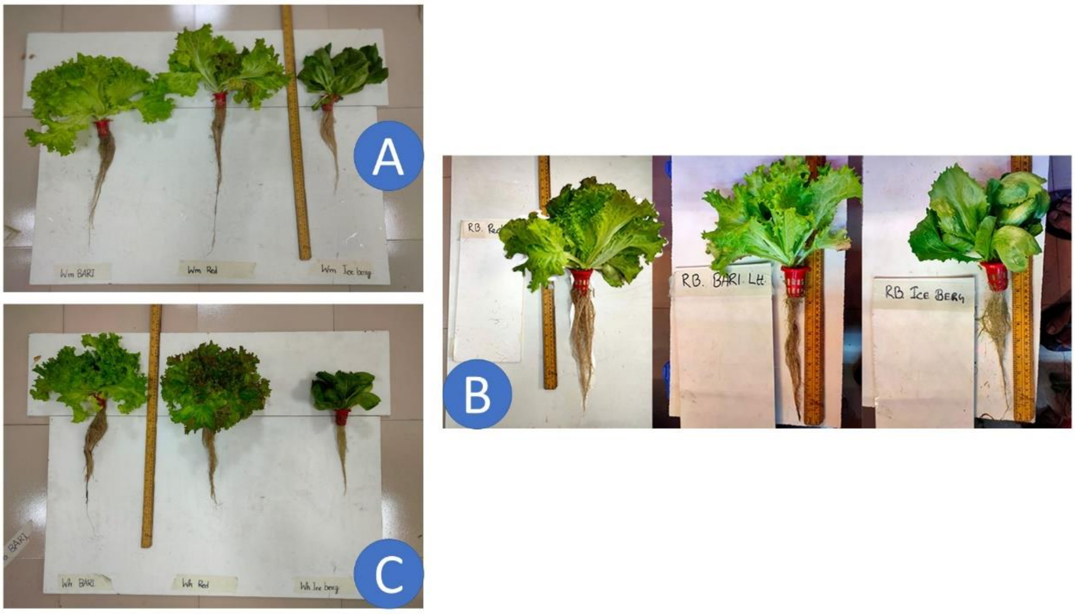

Plate 10: Growth comparisons on different light spectrum; were A. Influence of Warm (Wr) light for three lettuce varieties, B. Influence of Red:Blue (Rb-R4:B1) light for three lettuce varieties C. Influence of White Control (WhC) light for three lettuce varieties

Plate 11: Growth comparisons on different varieties over light spectrum; were A. Influence of BARI Lettuce (BL1) light for three different light spectrum, B. Influence of Ice Berg (IcB) light for three different light spectrum, C. Influence of Red Lettuce (RL) light for three different light spectrums

4.10 Yield pot^{-1} (kg)

4.10.1 Effect of light spectrum on yield pot^{-1} (kg)

Due to different light spectrum yields, pot^{-1} showed considerable variations. Maximum yield pot^{-1} was 0.74 kg, which was recorded from (Rb) treatment (Table 7, Appendix VII). On the other hand, the (WhC) control treatment gives the minimum yield pot^{-1}, which is 0.62 kg.

4.10.2 Effect of variety on yield pot^{-1} (kg)

The yield pot^{-1} exhibited remarkable variations due to the use of different lettuce varieties. Maximum yield pot^{-1} (0.72 kg) was recorded for the (IcB) Iceberg variety (Table 8, Appendix VII). In contrast, a minimum yield pot^{-1} (0.66 kg) was recorded for (RL) Red Lettuce.

4.10.3 Combined effect of different LED light spectrum and variety on yield pot^{-1} (kg)

Due to the combination of light spectrum and lettuce varieties, yield pot^{-1} showed significant variations (Table 9, Appendix VII). The combination of (RbIcB) Ice Berg and (Rb) light treatment produced the highest yield pot^{-1} (0.80 kg). On the other hand, white light treatment showed minimum yield pot^{-1} (0.59 kg) was recorded for Red Lettuce (WhCRL).

4.11 Yield (t ha^{-1})

4.11.1 Effect of light spectrum on yield (t ha^{-1})

Due to different light spectrum yields, ha^{-1} exhibited significant variations. maximum yield of 44.09 t h^{-1} (Table 7, Appendix VII), which was recorded from (Rb) treatment. While the (WhC) control treatment gives the minimum yield of 37.03 t h^{-1}.

4.11.2 Effect of variety on Yield (t ha^{-1})

Due to the use of different lettuce varieties, yield ha^{-1} exhibited significant variations. A maximum yield (42.86 t h^{-1}) (Table 8, Appendix VII) was recorded for the Iceberg variety. Red Lettuce (RL) produced a minimum yield of 39.00 t h^{-1}

4.11.3 Combined effect of different LEDs light and variety on Yield (t ha^{-1})

Significant variation was recorded for yield ha^{-1} due to interaction between the light spectrum and lettuce varieties. Rb light exhibited a maximum yield of ha^{-1} of 47.35 tons when combined with the Iceberg variety (RbIcB). While red lettuce combined gave a minimum yield of ha^{-1} (34.70 tons) under control treatment (WhCRL).

Table 7. Fresh weight leaf^{-1}, fresh weight plant^{-1}, dry weight, yield pot^{-1}, yield of lettuce influenced by LED light spectrum

Treatment	Fresh weight (g) leaf^{-1}	Fresh weight plant^{-1} (kg)	Dry weight (g)	Yield pot^{-1} (kg)	Yield (t ha^{-1})
Wh(control)	8.87 c	0.12 c	14.48 c	0.62 c	37.03 c
Rb	10.05 a	0.14 a	17.24 a	0.74 a	44.09 a
Wr	9.68 b	0.14 b	16.24 b	0.70 b	41.54 b
CV (%)					
LSD	0.28	0.001	0.92	0.04	2.35

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

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1

Table 8. Fresh weight leaf^{-1}, fresh weight plant^{-1}, dry weight, yield pot^{-1}, yield of lettuce influenced by varieties

Treatment	Fresh weight (g) leaf^{-1}	Fresh weight plant^{-1} (kg)	Dry weight (g)	Yield pot^{-1} (kg)	Yield (t ha^{-1})
BL1	9.84 a	0.13 ab	15.96 ab	0.69 ab	40.82 ab
RL	9.14 b	0.13 b	15.24 b	0.66 b	39.00 b
IcB	9.63 c	0.14 a	16.75 a	0.72 a	42.86 a
CV (%)					
LSD	0.28	0.001	0.92	0.04	2.35

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

Here, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

Table 9. Fresh weight leaf^{-1}, fresh weight plant^{-1}, dry weight, yield pot^{-1}, yield of lettuce influenced by LED light spectrum and varieties

Treatment	Fresh weight (g) leaf^{-1}	Fresh weight plant^{-1} (kg)	Dry weight (g)	Yield pot^{-1} (kg)	Yield (t ha^{-1})
WhCBL1	9.7 bc	0.127 de	14.69 de	0.63 de	37.58 de
WhCRL	7.8 e	0.118 e	13.57 e	0.59 e	34.70 e
WhCIcB	9.13 d	0.13 cd	15.18 cd	0.66 cd	38.82 c
RbBL1	10.53 a	0.141 c	16.30 bc	0.70 bc	41.70 bc
RbRL	9.8 b	0.14 b	16.90 b	0.73 b	43.23 b
RbIcB	9.83 b	0.16 a	18.51 a	0.80 a	47.35 a
WrBL1	9.3 cd	0.14 b	16.88 c	0.73 c	43.17 b
WrRL	9.83 b	0.13 cd	15.27 cd	0.66 cd	39.05 cd
WrIcB	9.93 b	0.144 bc	16.58 bc	0.72 bc	42.41 bc
CV (%)					
LSD	0.49	0.01	1.59	0.06	4.07

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

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

C. Quality parameters

4.12. Combined effect of different LED light spectrum and variety on organoleptic test

Marketable parameters such as crispness, sweetness, bitterness, sourness, and appearance of lettuce leaves influence its acceptability to consumers. These qualitative parameters were analyzed by an organoleptic test. A 25-member judge panel was created from among the students of Sher-e-Bangla Agricultural University, Dhaka. Samples of lettuce from different treatment combinations alone with a questionnaire (Appendix IX) were served among the judges in order to evaluate its acceptability. The results of the tests were summarized in (Table 10). The findings revealed that lettuce grown with the (RbIcB) treatment combination had the highest marketable quality with a score of 3133. In contrast, the lowest score (2905) was recorded in the treatment combination of (WrIcB). In terms of crispiness, the highest scoring treatment combination (RbIcB) received the highest score (684) while the lowest scoring treatment combination (WrIcB) received the lowest score (564).In respect of sweetness, products from (RbIcB) obtained the highest score (684), whereas the lowest score was recorded for WrIcB (556). Plants from WrRL and WrIcB treatment combinations had the highest score (632) and the lowest score (564), respectively, in terms of bitterness. Produce from WhCBL1 (667) obtained the highest sourness acceptance, while produce from RbBL (540) had the least acceptability based on sourness. The highest appearance score (643) was obtained by leaves from the (WrRL) treatment combination, whereas the least desirable appearance was found from the WrIcB (565). A prediction of the consumers' likings for different marketable qualities of the produced lettuce can be made based on the present results.

4.13. Color Measurement

4.13.1 Combined effect of different LED light spectrum and variety on color measurements

Remarkable variation was noted in the color of lettuce leaves, influenced by the combined effect of different LED light spectrums and lettuce varieties (Table 11 and Appendix VIII). The higher L* value indicates the lighter color, which was found in the treatment combination WhCBL1 (63.28) closely followed by treatment combinations WhCRL (61.35) and RbBL1(52.86) and the lower L* value found in the treatment combination WrIcB (34.57) closely followed by treatment combinations WhCIcB (35.83) and RbIcB (37.37). The redness value a* was highest in the treatment combination RbRL (37.10), closely followed by treatment combinations WrRL (35.17) and WhCRL (33.06), whereas the lowest a* value was found in the treatment combination RbIcB (7.42), which was statistically similar to the treatment combination WrIcB (7.92). The higher b* value indicates yellow color and was found in treatment combination WhCBL1 (69.01) and the lower value was found in treatment combination WhCIcB (18.63). The higher Chroma value was found in treatment combination WhCBL1 (69.81) and the lower value was found in treatment combination WhCIcB (20.48).

Table 10. Combined effect of different LED light spectrum and varieties on organoleptic attributes of lettuce leaves

Treatment	Crispness	Sweetness	Bitterness	Sourness	Appearance	Total
WhCBL1	604	592	612	667	584	3059
WhCRL	632	588	604	612	604	3040
WhCIcB	620	560	620	598	593	2991
RbBL1	568	632	620	604	604	3028
RbRL	580	604	624	564	632	3004
RbIcB	684	675	592	578	604	3133
WrBL1	656	665	598	540	602	3061
WrRL	592	567	632	632	643	3066
WrIcB	564	556	564	656	565	2905

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

Highly Acceptable (HA=7), Slightly Acceptable (SA=5) and Not acceptable (NA=2)

Table 11. Combined effect of different LED light spectrum and varieties on chromatic characteristics of the lettuce leaves

Treatment combinations	L^*	a^*	b^*	Chroma
WhCBL1	63.28 a	10.55 f	69.01 a	69.81 a
WhCRL	61.35 b	33.06 c	26.80 d	42.55 d
WhCIcB	35.83 g	8.51 g	18.63 h	20.48 h
RbBL1	52.86 c	17.36 d	57.55 b	60.11 c
RbRL	41.06 d	37.10 a	54.73 c	66.11 b
RbIcB	37.37 f	7.42 h	21.03 g	22.30 gh
WrBL1	38.83 e	15.57 e	25.15 e	29.57 e
WrRL	37.62 f	35.17 b	23.66 f	42.38 de
WrIcB	34.57 h	7.92 gh	21.50 g	22.91 g
CV%	1.37	3.12	1.06	1.05
$LSD_{(0.05)}$	1.06	1.05	0.65	0.78

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

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

4.14 SPAD Value

4.14.1 Effect on different LED light spectrum on SPAD Value

Due to the various light spectrums, SPAD expressed remarkable variation at 7, 14, 21, 28, and 35 DAT (Figure 10, Appendix VIII). The highest SPAD was at 22.411, 23.909, 21.417, 23.702, and 27.653 for lettuce plants under (Rb) light treatment at 7, 14, 21, 28, and 35 DAT respectively. On the contrary, the lowest SPAD of 17.789, 21.789, 18.122, 21.428, and 24.3 was recorded under the (Wr) warm light spectrum for all the observed DAT.

4.14.2 Effect on different varieties on SPAD Value

Due to the use of different lettuce varieties, SPAD showed significant variation at 7, 14, 21, 28, and 35 DAT (Figure 11, Appendix VIII). Iceberg lettuce (IcB) presented the highest SPAD of 32.378, 36.314, 31.317, 34.637, and 40.409 for all observed DAT. Whereas, minimum SPAD of 12.839, 15.394, 12.694, 15.81, and 17.189 were observed for (RL) Red lettuce at 7, 14, 21, 28, and 35 DAT respectively.

4.14.3 Combined effect of different varieties and LED light spectrum on SPAD Value

Due to interaction between the light spectrum and lettuce varieties, a remarkable variation was observed for the SPAD of lettuce (Figure 12, Appendix VIII). Red lettuce expressed the lowest SPAD at 12.183, 14.2 and 13.358 under the Red-Blue light spectrum at 7, 14 and 35 DAT, respectively, while control treatment on (WhCBL1) BARI lettuce 1 gave a minimum SPAD of 11.583 at 21 DAT. Moreover, at 28 DAT, the shortest SPAD (14.217) was recorded from (RbBL1) BARI lettuce 1 under Rb light treatment. On the contrary, the highest SPAD of 39.883, 41.077, 36.82, 42.957, and 44.35 was observed for Iceberg at 7, 14, 28, and 35, at harvest 42 DAT respectively, under the (RbIcB) treatment combination, while the same variety showed the highest SPAD of 37.20, at 42 DAT under the (WhC) control treatment.

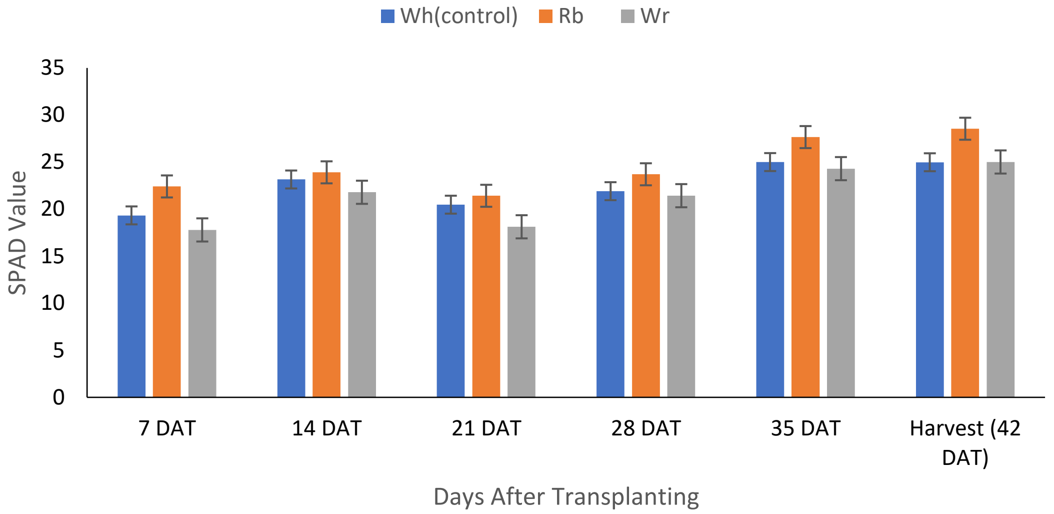

Figure 10: SPAD Value of lettuce leaves as influenced by different LED light spectrum at different days after transplanting

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1

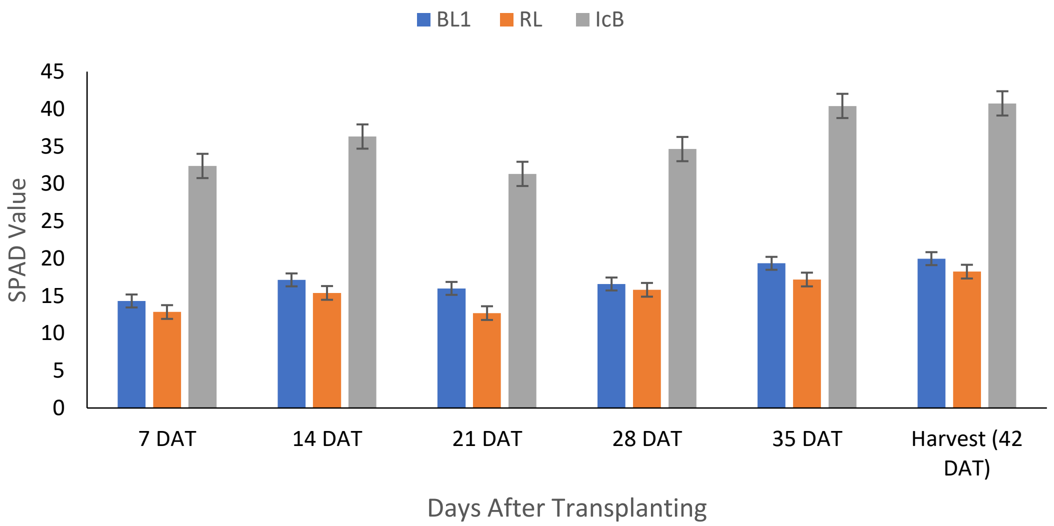

Figure 11: SPAD Value of lettuce leaves as influenced by different lettuce varieties at different days after transplanting

Here, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

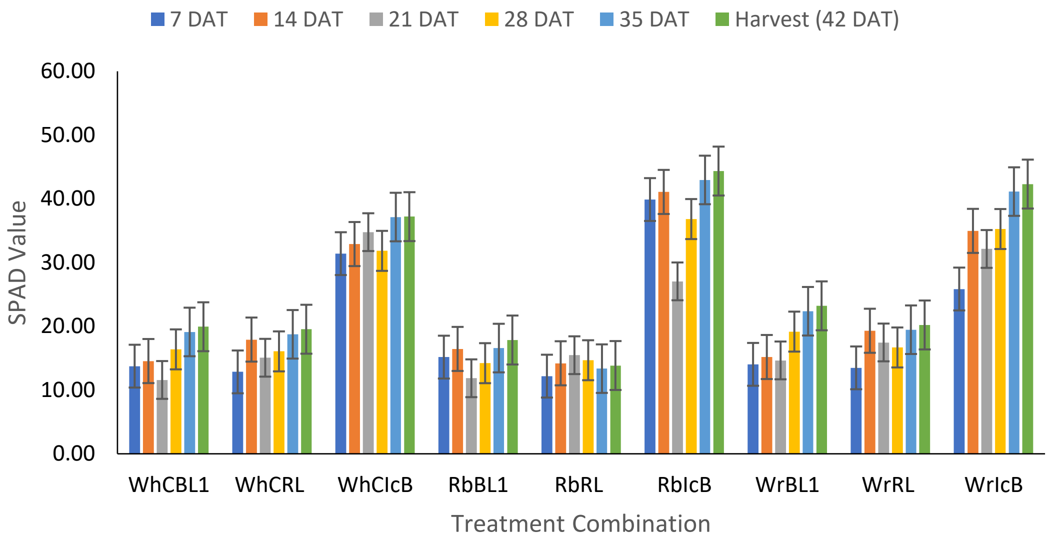

Figure 12: SPAD Value of lettuce leaves as influenced by different LED light spectrum and different varieties at different days after transplanting

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

4.15 Total soluble solid (ºBrix)

4.15.1 Effect of light spectrum on total soluble solid (ºBrix)

The Brix percentage present in lettuce plants expressed statistically highly significant variation due to different light spectrums. The highest Brix percentage was observed at Rb (4.92) under light treatment. whereas the lowest brix (3.95) was recorded under control treatment (WhC). (Table 12, Appendix VIII)

4.15.2 Effect of variety on total soluble solid (ºBrix)

Due to use of different varieties of lettuce highly significant variation was recorded for Brix percentage of lettuce plant (Table 13, Appendix VIII). Ice Berg lettuce exhibited highest percentage of brix (5.82 %). In contrast, Brix was found lowest in BL1 (3.77) from BARI Lettuce-1 variety.

4.15.3 Combined effect of different LED light spectrum and varieties on total soluble solid (ºBrix)

Brix percentage varied significantly due to interactions between light spectrum and lettuce varieties. Due to the Wr light treatment, brix percentage was recorded at a higher value (6.70) for the WrIcB treatment combination (Table 14 Appendix VIII), while the control treatment presented the lowest brix percentage (3.43) as similar results were shown for WhCBL1 (3.56) for Red lettuce and BARI Lettuce 1 variety, respectively.

4.16 Vitamin-C content (mg. 100g^{-1} FW)

4.16.1 Effect of light spectrum on vitamin-C content (mg 100g^{-1} FW)

Significant variation was observed for vitamin C present in lettuce plant due to different light spectrum. The content of vitamin C under both Wr and control treatment (WhC) was noted to be higher, which was 0.96 mg per 100gm of lettuce, whereas the minimum vitamin C (0.93 mg/100gm of lettuce) was recorded from the (Rb) treatment (Table 12, Appendix VIII).

4.16.2 Effect of variety on vitamin-C content (mg 100g^{-1} FW)

Vitamin C exhibited remarkable variation due to the use of different varieties of lettuce. Iceberg showed the highest vitamin C content (1.08 mg 100g^{-1} FW). BARI lettuce-1 had the lowest vitamin C content of 0.75 mg/100gm (Table 12, Appendix VIII). According to (Mou 2005), they have also observed this interdependence and reported a higher vitamin C content in leaf lettuce than in head-forming types. This proved to be true in our study as well. Leaf lettuces Levistro (9.60mg/100g) and Kibou (5.25mg/100g) contain more vitamin C than head lettuce Butterhead (3.85mg/100g).

4.16.3 Combined effect of different LED light spectrum and variety on vitamin-C content (mg 100g^{-1} FW)

Vitamin C expresses significant variation due to the interaction between the light spectrum and lettuce varieties. The Vitamin C content in the Iceberg variety showed the maximum result, which was 1.20 mg/100 gm under the (RbIcB) treatment combination. Under the Rb light spectrum (RbBL1), the BARI lettuce-1 variety had a minimum vitamin C content of 0.7 mg/100gm (Table 14, Appendix VIII).

4.17 Anthocyanin (mg 100g$^{-1)}$

4.17.1 Effect of light spectrum on anthocyanin (mg 100g$^{-1)}$

Anthocyanin in lettuce plants exhibited statistically significant variation due to different light spectrums. Anthocyanin was observed at high (6.54 mg/100gm) for (Rb) light and low (3.90 mg/100gm) under (WhC) control treatment (Table 12, Appendix VIII).

4.17.2 Effect of variety on anthocyanin (mg 100g^{-1})

Anthocyanin in lettuce plants showed significant variation due to the use of different varieties of lettuce. Red lettuce presented the maximum result for anthocyanin, which was 6.66 mg/100 gm (Rb). While (BL1) BARI lettuce 1 exhibited the lowest anthocyanin and antioxidant percentage of any lettuce plant, which is 2.83 mg/100 gm (Table 13, Appendix VIII).

4.17.3 Combined effect of different LED light spectrum and variety on anthocyanin (mg. 100g$^{-1)}$

Anthocyanin was found to express a wide range of variation due to the interaction between the light spectrum and lettuce varieties. The highest anthocyanin was recorded at 9.99 mg/100gm for Red Lettuce under control treatment (WhCRL) and the minimum was found at 2.73 mg/100gm for BARI Lettuce-1 under warm treatment (WrBL1) (Table 14, Appendix VIII).

4.18 Antioxidant activity (%)

4.18.1 Effect of light spectrum on antioxidant activity (%)

Antioxidants present in lettuce plants presented significant variation due to different light spectrums (Table 12, Appendix VIII). The percentage of antioxidant was found to be highest (54.23%) in the warm light spectrum (Wr) and lowest (37.84%) in the control treatment (WhC).

4.18.2 Effect of variety on antioxidant activity (%)

Due to the use of different varieties of lettuce, considerable variation was recorded for the antioxidant percentage of the lettuce plant (Table 13, Appendix VIII). (RL) Red lettuce presented the maximum result for antioxidant percentage, which is 50.64 %. whereas (BL1) BARI Lettuce-1 produced the lowest antioxidant percentage of lettuce plants (41.55%).

4.18.3 Combined effect of different LED light spectrum and variety on antioxidant activity (%)

Due to the interaction between the light spectrum and lettuce varieties, antioxidants exhibited significant variation. The control treatment had the highest percentage of antioxidants, 60.54% for Red Lettuce (WhCRL), with a nearly similar result (55.21%) under this RbIcB treatment combination. The lowest antioxidant percentage of 32.50 % was observed for BARI Lettuce-1 under control treatment (WhCBL1) (Table 14, Appendix VIII). According to Huang *et al*. (2021) the contents of vitamin C, flavonoids and anthocyanins in two-leaf-color lettuce were significantly increased, but no significant differences were observed in vitamin C, flavonoids and anthocyanins among the treatment similar to what was found for the anthocyanin content of green-leaf lettuce. However, the content of anthocyanins in red-leaf lettuce gradually increased with increasing time intervals, with the highest content being found in Rb light. Supplemental alternating red and blue light slightly increased the antioxidant capacity [1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging rate and antioxidant power], but no significant differences were observed other treatment.

Table 12. ºBrix, Vitamin-C Content (mg. 100g^{-1} FW), Anthocyanin (mg. 100g^{-1} FW), Antioxidant %, of lettuce influenced by LED light spectrum

Treatment	(ºBrix)	Vitamin-C Content (mg. 100g^{-1} FW)	Anthocyanin (mg. 100g^{-1} FW)	Antioxidant (%)
Wh(control)	4.95 b	0.96 a	3.90 c	37.84 c
Rb	4.78 b	0.93 a	6.54 a	49.16 a
Wr	8.38 a	0.96 a	4.43 b	54.23 b
CV (%)	6.99	10.13	10.68	3.03
LSD	0.32	0.09	0.52	0.66

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

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1

Table 13. ºBrix, Vitamin-C Content (mg. 100g^{-1} FW), Anthocyanin (mg. 100g^{-1} FW), Antioxidant %, of lettuce influenced by varieties

Treatment	(ºBrix)	Vitamin-C Content (mg. 100g^{-1} FW)	Anthocyanin (mg. 100g^{-1} FW)	Antioxidant (%)
BL1	7.80 a	0.75 b	2.83 c	41.55 c
RL	4.58 c	1.02 a	6.66 a	50.64 a
IcB	5.74 b	1.08 a	5.38 b	49.03 b
CV (%)	6.99	10.13	10.68	3.03
LSD	0.32	0.09	0.52	0.66

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

Here, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

Table 14. ºBrix, Vitamin-C Content (mg. $100g^{-1}$ FW), Anthocyanin (mg. $100g^{-1}$ FW), Antioxidant %, of lettuce influenced by LED light spectrum and varieties

Treatment	(ºBrix)	Vitamin-C Content (mg. $100g^{-1}$ FW)	Anthocyanin (mg. $100g^{-1}$ FW)	Antioxidant (%)
WhCBL1	3.56e	0.76 c	2.80 e	32.50 f
WhCRL	3.43 e	0.96 b	9.99 a	60.54 a
WhCIcB	4.86c	1.06 ab	6.83 b	54.45 b
RbBL1	3.86de	0.70 c	2.97 e	53.19 c
RbRL	4.90 c	1.00 b	5.45 c	54.28 bc
RbIcB	5.90 b	1.20 a	4.87 cd	55.21 b
WrBL1	3.90 de	0.78 c	2.73 e	38.96 d
WrRL	4.16 d	1.1 ab	4.53 cd	37.11 e
WrIcB	6.70 a	1.00 b	4.44 d	37.44 e
CV (%)	6.99	10.13	10.68	3.03
LSD	0.55	0.16	0.91	1.14

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

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

Economical Analysis

Input costs for indoor land preparation, nutrients, labor, and operation costs required for all the intercultural operations such as from seed sowing to harvesting of lettuce were recorded for each unit plot and converted into cost Tk/ha (Appendix X). The price of lettuce was considered as per the market rate during the off season. The economic analysis was presented under the following headings:

Gross return (Tk ha^{-1})

The combination of different LEDs and varieties showed different values in terms of gross return under the trial (Table 15 and Appendix X). The highest gross return (Tk. 7102500) was obtained from the treatment combination RbIcB and the second highest gross return (Tk. 6484500) was found in RbRL. The lowest gross return (Tk. 5205000) was obtained from WhCRL.

Net return (Tk ha^{-1})

In the case of net return, different treatment combinations showed different levels of net return under the present trial (Table 15 and appendix X). The highest net return (Tk. 3923500) was found from the treatment combination WrBL1, and the second highest net return (Tk. 3809500) was obtained from the combination WrIcB. The lowest (Tk. 2635675) net return was obtained by WhCRL.

Benefit cost ratio (BCR)

In all treatment combinations, the benefit cost ratio was different when different LEDs and varieties were combined (Table 15 and Appendix X). The highest benefit cost ratio (3.38) was recorded in WrBL1. The lowest benefit-cost ratio (2.20) was obtained from RbBL1. From the economic point of view, it is apparent that the WrBL1 treatment combination was the most profitable one compared to the rest of the treatment combinations under the study.

Table 15. Cost and return of lettuce production as influenced by different LED light spectrum and varieties in indoor farming

Treatment Combination	Cost of Production (tk ha^{-1})	Yield of lettuce (t ha^{-1})	Gross return (tk ha^{-1})	Net return (tk ha^{-1})	Benefit cost ratio (BCR)
WhCBL1	2569325	37.58	7516000	4946675	2.93
WhCRL	2569325	34.70	6940000	4370675	2.70
WhCIcB	2569325	38.82	7764000	5194675	3.02
RbBL1	3794499	41.70	8340000	4545500	2.20
RbRL	3794499	43.23	8646000	4851500	2.28
RbIcB	3794499	47.35	9470000	5675500	2.50
WrBL1	2552000	43.17	8634000	6082000	3.38
WrRL	2552000	39.05	7810000	5258000	3.06
WrIcB	2552000	42.41	8482000	5930000	3.32

Here, WhC=White Light (Control); Wr = Florescent Light (known as Warn light); Rb = Red and Blue - R4:B1, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

CHAPTER V

SUMMARY AND CONCLUSION

Regarding the growth parameters such as plant height, number of leaves, leaf length, leaf breadth, SPAD value at 7, 14, 21, 28, 35 DAT and harvest time observed, the tallest plant was 7.52 cm, 10.90 cm, 12.67 cm, 19.17 cm, 27.33 cm, and 30.06 cm, respectively for (RbBL1) BARI Lettuce-1 in (Rb) light treatment. On the contrary, the (IcB) Iceberg variety of lettuce presented the smallest plants at 5.93 cm, 7.07 cm, 7.33 cm, and 7.67 cm at 7, 14, 21, and 28 DAT, respectively, in Wr spectrum treatment. The highest observed leaf number (6.00, 6.66, 11.00, and 13.00) in (RbBL1) light treatment at 7, 14, 21, and 28 DAT, respectively, was at 7, 14, 21, and 28 DAT. Moreover, (RbRL) Red Lettuce exhibits a maximum number of leaves of 16.33 at 35 DAT. On the contrary, the number of leaves was found to be lowest at 4.00 for both (WhCRL, WrIcB) Red lettuce and Ice Burg in white and warm light, respectively, at 7 DAT, and only Iceberg showed lower leaves at 4.00 at 14 DAT in (WrIcB) warm light. Longest leaf length of 6.75 cm, 10.67 cm, 17.00 cm, 24.67 cm, and 27.41 cm were observed for (RbBL1) at 7, 14, 21, 28, and 35 DAT and harvest time, respectively. The shortest leaf lengths for (RbIcB) were 4.69 cm, 5.08 cm, and 5.83 cm at 7, 14, and 21DAT, respectively. Leaf breath BARI Lettuce-1 exhibited the highest breadth at 8.50 cm, 11.67 cm, 16.17 cm, and 17.96 cm under fluorescent light conditions at 21, 28, and 35 DAT and harvest time, respectively. On the other hand, (IcB) Iceberg at 7 DAT showed the smallest breadth of 1.32 cm under warm light treatment, while the shortest breadth of 1.67 cm, 2.17 cm, 2.50 cm, 9.83 cm, and 10.93 cm for other observed DAT was recorded under (WhC) white light treatment. Fresh weight plant^{-1} was influenced by lettuce varieties Iceberg exhibited the highest fresh weight plant^{-1} (0.16 kg) due to the (Rb) light treatment. RL Red Lettuce had the lowest fresh weight plant-1 in the control treatment, which was 0.118 kg. Yield plot^{-1} showed significant variations where maximum yield plot^{-1} (0.80 kg) was observed for (RbIcB) Iceberg was due to the (Rb) light treatment while, due to the white light treatment, minimum yield plot^{-1} (WhCRL) (0.59 kg) was recorded for Red Lettuce. Rb light exhibited a maximum yield of ha^{-1} (47.35 tons) for the Iceberg variety (WrIcB). While red lettuce was recorded to give a minimum yield of ha^{-1} 34.70 tons under control treatment. For the organoleptic test, the findings revealed that lettuce grown with the (RbIcB) treatment combination had the highest marketable quality with a score of 3133. In contrast, the lowest score (2905) was recorded in the treatment combination of (WrIcB). The Brix percentage exhibited significant variations due to the warm light treatment. As high as WrIcB (6.70%) for Iceberg lettuce, while control treatment presented the lowest brix percentage, WhCRL (3.43%), as similar results were shown for WhCBL1 (3.56%) for Red lettuce and BARI Lettuce 1 variety, respectively. The Vitamin C content in the Ice Berg variety showed the maximum result, which was 1.20 mg/100 gm under red blue light (RbIcB). Whereas, a minimum vitamin C of 0.7 mg/100 gm was found for the BARI Lettuce 1 variety under the Red-Blue light spectrum (RbBL1). Anthocyanin levels were measured at 9.99 mg/100gm for Red lettuce under control treatment (WhCRL) and a minimum of 2.73 mg/100gm for BARI lettuce 1 under warm treatment (WrBL1). The antioxidant percentage for red lettuce (WhCRL) was 60.54%. The lowest antioxidant percentage of 32.50 % was observed for BARI lettuce 1 under control treatment (WhCBL1).

From the above results, it can be concluded that among the treatments of different LED-light spectrum in vertical farming, the treatment (RbIcB) light and the Iceberg variety had the most significant positive effect on growth, yield contributing parameters, and yield and quality parameters of lettuce and resulted in the highest fresh weight plant^{-1} (0.16 kg) and total yield (47.35 t ha^{-1}) compared to all other treatments.

Recommendations

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

1. Some other LED-light spectrum treatments can be used in future study.
2. Another variety of lettuce need to be considered before final recommendation.

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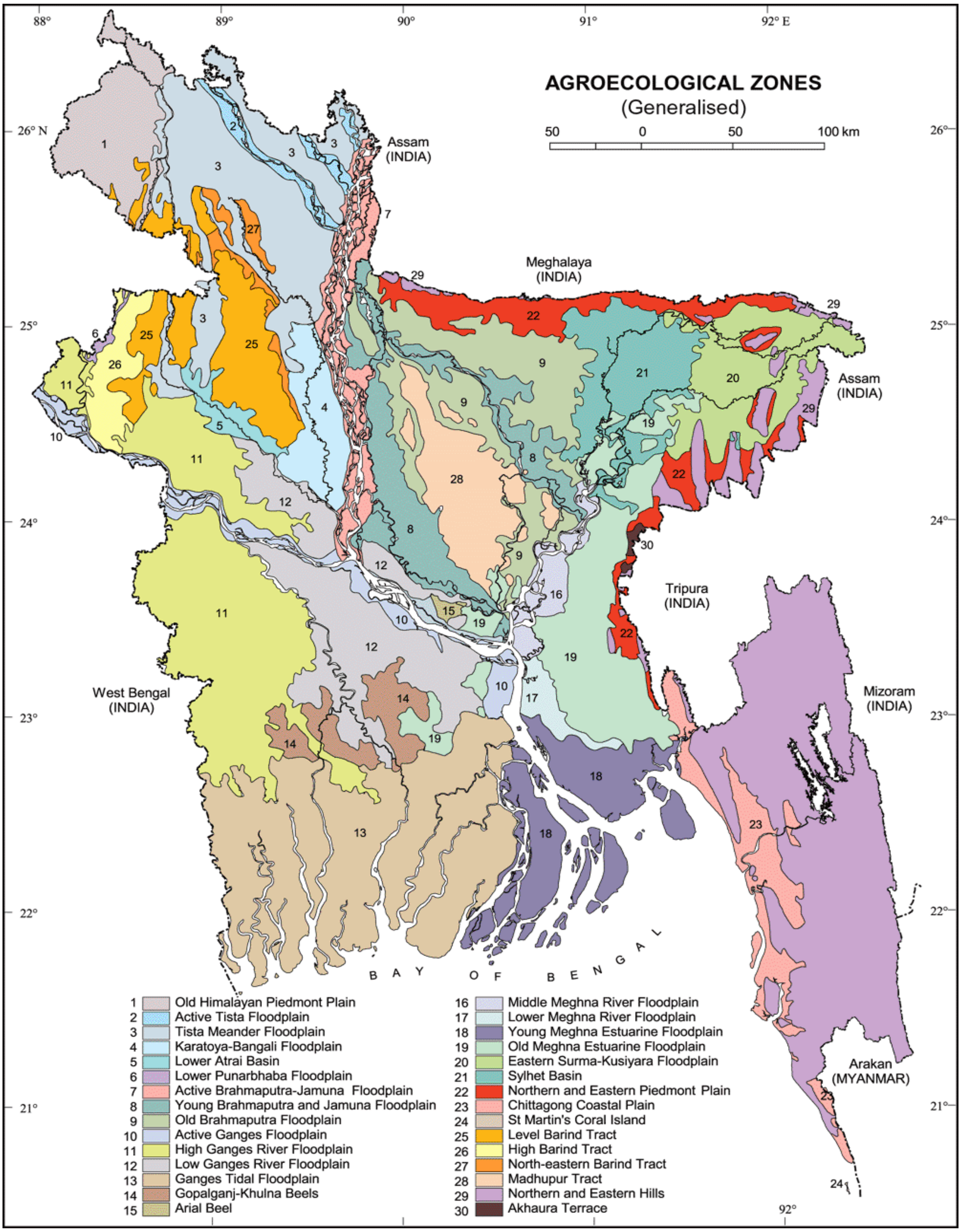
APPENDICES

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



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

Month	Air temperature (ºc)		Relative humidity (%)	Rainfall (mm) (total)	Sunshine (hr)
	Maximum	Minimum			
July, 2020	35	27	93	227	5.8
October, 2020	29.6	21.5	89	0	7.9

Source: Bangladesh Meteorological Department (Climate and Weather Division), Agargoan, Dhaka – 1212

Appendix III. Layout of the experiment treatments

R1	WhC BL1	WhC RL	WhC IcB
	WrBL1	WrRL	WrIcB
	RbBL1	RbRL	RbIcB
R2	WhC BL1	WhC RL	WhC IcB
	WrBL1	WrRL	WrIcB
	RbBL1	RbRL	RbIcB
R3	WhC BL1	WhC RL	WhC IcB
	WrBL1	WrRL	WrIcB
	RbBL1	RbRL	RbIcB

Legend

Treatments

Factor A: Light Spectrum- 100 umol $m^{-2}s^{-1}$

1. WhC=White Light (Control)
2. Wr = Florescent Light
3. Rb = Red and Blue - R4:B1

Factor B: Lettuce Variety

a. BL1- BARI Lettuce 1
b. RL- Red Lettuce
c. IcB- Ice Burg Lettuce

Appendix IV: Analysis of variance of the data on plant height and number of leaves plant^{-1} at different DAT of lettuce as influenced by different LED light spectrum and varieties

Source of Variance	Degree of Freedom (DF)	Mean Square											
		Plant Height (cm)						Number of leaves plant^{-1}					
		7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	Harvest	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	Harvest
Replication	2	2.20	0.50	1.33	1.41	8.94	16.41	1.40	0.48	6.48	4.48	5.44	6.37
Light Spectrum (A)	2	2.41**	0.75**	1.54**	36.35**	86.67**	112.93*	3.85*	3.37**	1.81**	2.70**	7.00^{NS}	6.3704^{NS}
Variety (B)	2	4.23**	20.70**	40.77**	98.98**	138.70**	154.17**	3.62*	0.70^{NS}	21.14**	21.37**	37.00**	38.37**
A X B	4	1.72^{NS}	0.88**	0.48^{NS}	8.24**	12.90**	14.92*	3.92^{NS}	1.42**	0.42^{NS}	0.59^{NS}	2.33^{NS}	2.25^{NS}
Error	16	2.71	0.10	0.29	1.00	2.90	4.68	7.25	0.36	0.27	0.44	2.65	2.91

**: Significant at 0.01 level of probability; *: Significant at 0.05 level of probability; NS: Non significant

Appendix V: Analysis of variance of the data on leaf length and leaf breadth at different DAT of lettuce as influenced by different LED light spectrum and varieties

Source of Variance	DF	Mean Square											
		Leaf Length (cm)						Leaf Breadth (cm)					
		7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	Harvest	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	Harvest
Replication	2	2.05	0.55	2.80	1.46	16.46	20.32	2.06	0.52	0.38	0.09	5.92	7.31
Light Spectrum (A)	2	1.13^{NS}	0.60^{NS}	0.50^{NS}	20.44^{**}	81.85^{**}	101.04^{**}	14.72**	3.63**	0.57**	5.21**	27.12**	33.48**
Variety (B)	2	10.55**	5.92^{**}	43.58**	92.43**	148.29**	183.07**	49.3**	31.67**	82.84*	123.25**	31.66**	39.08**
A X B	4	1.45^{NS}	0.45^{NS}	0.31^{NS}	8.14**	9.00^{NS}	11.12^{NS}	4.37^{NS}	2.13^{NS}	0.01^{NS}	1.68^{NS}	1.69^{NS}	2.09^{NS}
Error	16	4.18	0.57	0.28	1.05	3.53	4.36	19.35	0.91	0.15	0.60	3.26	4.03

**: Significant at 0.01 level of probability; *: Significant at 0.05 level of probability; NS: Non significant

Appendix VI: Analysis of variance of the data on SPAD Value at different DAT of lettuce as influenced by different LED light spectrum and varieties

Source of Variance	DF	Mean Square					
		SAPD Value					
		7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	Harvest
Replication	2	15.75	3.53	9.20	4.55	16.82	17.43
Light Spectrum (A)	2	49.83 *	10.38^{NS}	25.90*	12.94^{NS}	28.15^{NS}	28.10^{NS}
Variety (B)	2	1065.23**	1212.59**	888.47**	1021.16**	1480.84**	1484.02*
A X B	4	51.49**	33.76^{NS}	16.93^{NS}	14.10^{NS}	28.48^{NS}	28.43^{NS}
Error	16	14.46	23.17	7.31	23.66	29.84	27.86
**: Significant at 0.01 level of probability; *: Significant at 0.05 level of probability; NS: Non significant							

Appendix VII: Analysis of variance of the data on tap root length, shoot diameter, fresh weight leaf^{-1}, fresh weight plant^{-1} dry weight, yield pot^{-1}, yield as influenced by different LED light spectrum and varieties

Source of Variance	DF	Mean Square						
		Tap Root Length (cm)	Shoot Diameter (cm)	Fresh weight (g) leaf^{-1}	Fresh weight plant^{-1} (kg)	Dry weight (g)	Yield Pot^{-1} (kg)	Yield (t ha^{-1})
Replication	**2**	1.94	0.03	0.34	0.0000	0.04	0.00	0.29
Light Spectrum (A)	**2**	0.26^{NS}	0.25**	3.26**	0.001**	17.58**	0.03**	114.99**
Variety (B)	**2**	3.79*	0.13*	1.16**	0.0003**	5.13**	0.009*	33.60*
A X B	**4**	1.60^{NS}	0.01^{NS}	1.27**	0.0001^{NS}	1.51^{NS}	0.002^{NS}	9.88^{NS}
Error	**16**	1.03	0.03	0.08	0.0001	0.85	0.00	5.55
**: Significant at 0.01 level of probability; *: Significant at 0.05 level of probability; NS: Non significant								

Appendix VIII: Analysis of variance of the data on brix (%), vitamin-C Content (mg. 100g^{-1} FW), anthocyanin (mg. 100g^{-1} FW), antioxidant (%), L*, a*, b*, Chroma as influenced by different LED light spectrum and varieties

Source of Variance	DF	Mean Square							
		Brix (%)	Vitamin-C Content (mg. 100g^{-1} FW)	Anthocyanin (mg. 100g^{-1} FW)	Antioxidant (%)	L*	a*	b*	Chroma
Replication	2	0.65	0.02	0.22	0.34	0.02	0.49	0.1	0.47
Light Spectrum (A)	2	2.71**	0.002^{NS}	17.50**	3.26**	72.21**	36.6**	10.6.34**	143.54**
Variety (B)	2	10.60**	0.29**	34.11**	1.16**	1432.27**	1808.63**	4309.18**	2915.98**
A X B	4	0.76*	0.02^{NS}	6.49**	1.27**	17.65**	6.98**	38.67*	37.92**
Error	16	0.10	0.01	0.28	0.08	0.35	0.36	0.146	0.27

**: Significant at 0.01 level of probability; *: Significant at 0.05 level of probability; NS: Non significant

Appendix IX: Questionnaire for quality attributes assessment of lettuce

Treatment	Crispness			Sweetness			Sourness			Bitterness			Appearance		
	HA	SA	NA	HA	SA	NA	HA	SA	NA	HA	SA	NA	HA	SA	NA
WhCBL1															
WhCRL															
WhCIcB															
RbBL1															
RbRL															
RbIcB															
WrBL1															
WrRL															
WrIcB															

Here, HA $\Rightarrow$ Highly Acceptable (7), SA $\Rightarrow$ Slightly acceptable (5) and NA$\Rightarrow$ Not Acceptable

Name of the Judge:

Address:

Age:

Profession:

Signature: Date:

Appendix X: Per hectare production cost of lettuce under exposed of LED light spectrum in indoor farming condition

A. **Input Cost**

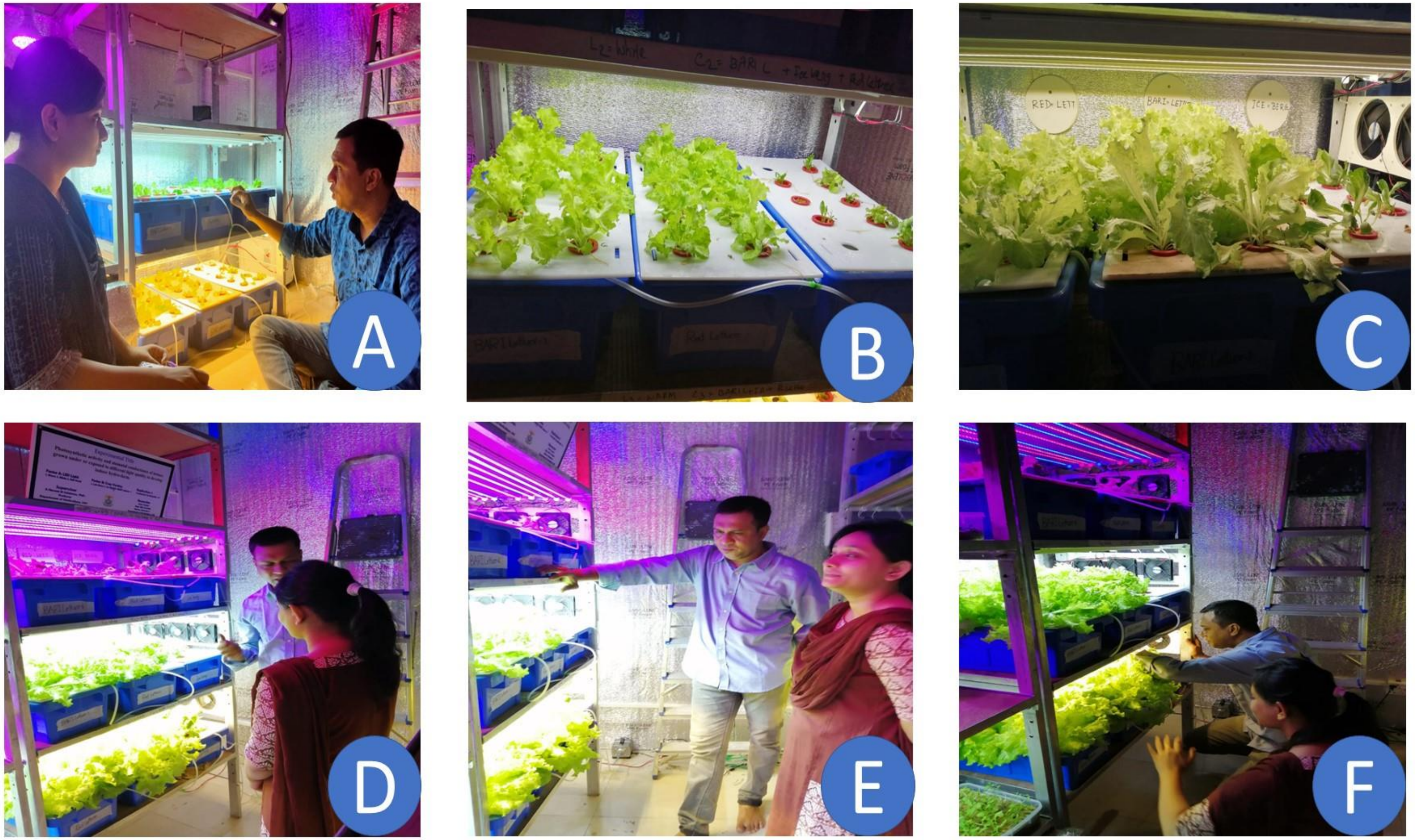
Treatment Combination	Labor cost (tk.)	Seed Cost (tk)	Nutrient Cost (tk)	Light Cost (tk)	Operational cost (tk)	Total Input (A) Cost (tk)
WhCBL1	480000	10000	500000	666500	450000	2106500
WhCRL	480000	10000	500000	666500	450000	2106500
WhCIcB	480000	10000	500000	666500	450000	2106500
RbBL1	480000	10000	500000	1733333	550000	3273333
RbRL	480000	10000	500000	1733333	550000	3273333
RbIcB	480000	10000	500000	1733333	550000	3273333
WrBL1	480000	10000	500000	600000	500000	2090000
WrRL	480000	10000	500000	600000	500000	2090000
WrIcB	480000	10000	500000	600000	500000	2090000

Here, WhC=White Light (Control); Wr = Warm Light; Rb = Red and Blue - R4:B1, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce

B. Overhead cost

Treatment Combination	Cost of lease of land for 6 months (13% of value of land Tk. 45,00000/year	Miscellaneous cost (Tk. 5% of the input cost)	Interest on running capital for 1 year (Tk.13% of cost 10,00000/year)	Sub Total (B)	Total cost of production (Tk./ha) [Input cost (A)+ overhead cost (B)]
WhCBL1	292500	105325.00	65000	462825	2569325.00
WhCRL	292500	105325.00	65000	462825	2569325.00
WhCIcB	292500	105325.00	65000	462825	2569325.00
RbBL1	292500	163666.65	65000	521166.65	3794499.65
RbRL	292500	163666.65	65000	521166.65	3794499.65
RbIcB	292500	163666.65	65000	521166.65	3794499.65
WrBL1	292500	104500.00	65000	462000	2552000.00
WrRL	292500	104500.00	65000	462000	2552000.00
WrIcB	292500	104500.00	65000	462000	2552000.00

Here, WhC=White Light (Control); Wr = Warm Light; Rb = Red and Blue - R4:B1, BL1=BARI Lettuce-1, RL=Red Lettuce, IcB=Ice Berg Lettuce



Appendix XI: Supervisor visit in experimental areas; were A. Discussion about light intensity and PPDF (7 DAT), B Growth status of Control light (14 DAT), C. Growth status of Warm light (21 DAT) . D. Air flow and Temp. checking (21DAT), E. Direction from supervisor for fixing light PPDF and Temp (21 DAT), F. Checking the growth by supervisor (35DAT)