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Influence of Substrate and Supplementary LED lighting on Vertical Farming of Basil (*Ocimum basilicum* L.) and Pak Choi (*Brassica rapa* var. chinensis)

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Authors' contributions

This work was carried out in collaboration among all co-authors. Authors SHW and NKGKRM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors WAPW, CSLMK and BK managed the analyses of the study. Author NKGKRM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Application of "plant factory" concept in protected culture is gaining momentum due to its technological and economic merits in many countries. This research examined the plant growth and yield of vertically grown pak choi (*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 coco-peat 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

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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 pak choi was achieved by the normal LEDs. Horticulture graded to LED maintained fairly high chlorophyll a and b contents contributing to its characteristic leaf color.

Keywords: NFT; growth; basil; light; pakchoi.

1. INTRODUCTION

Recent advancement in agro-technology, knowledge on plant growth and market developments of agric produce have immensely contributed to the development of plant factories as an economically viable solution for producing high quality low-grown vegetables and herbs. When consider about herbs, basil (*Ocimum basilicum* L.), which belongs to family Lamiaceae has a great market demand as it is an essential ingredient in Western cuisine. Moreover, basil is also cultivated for fresh market consumption as well as for cosmetic and pharmaceutical uses especially because basil oil is known to possess several antimicrobial activities [1]. Research evidences clearly show its variable growth performances in different growing systems. Meanwhile, vertical farming or "plant factories" usually practiced the nutrient film technique (NFT) of basil while some growers grow them in compost based substrates [2]. Further to this, coir dust (coco-peat) is a widely popular plant growing medium in protected culture (Desa, 2017). It is a cheap and renewable source, having a high water and nutrient holding capacities. Further when it is mixed with compost, can suppress growth of plant nutrients are supplemented while soil borne pathogens are suppressed. As a result, compost supplemented coir substrate enhances the yield and quality of the final product of baby leafy vegetables while positively influencing the polyphenolic compound content [3]. Hence, hydroponics culture with coco-peat, compost and various other non-soil substrates have immersed as an alternative for conventional soil based vegetable cultivation, despite its enormous economic and environmental merits [4]. However NFT (with fully hydroponics fertigation) has become the first choice of vertical farming.

Pak Choi (*Brassica rapa* var. chinensis) is one of the most popular members of the oriental group of leafy vegetables which belong to *Brassicacese* family. It is grown successfully under protected environments. Light is a major limiting factor on the productivity of greenhouse vegetables during a prolonged series of cloudy days, especially in the winter. Recent technological advances in protected culture in artificial light sources have introduced Light Emitting Diodes (LED) [5]. It reported a great success among growers during last few years as it is a promising greenhouse lighting solution, over traditional lighting sources [6]. The main advantage of LEDs over all other lamp types is its energy saving ability [6]. Other than that, fast switching, higher durability, longer life time, lower thermal radiation, narrow variation in specific wavelength for targeted crops, etc. make them more applicable for protected culture [7]. The LEDs have become the best choice for highly intensive forms of vertical farming as a viable artificial lighting solution.

Meanwhile too much of nitrates in food are undesirable for human consumption. Past research evidences support that increased nitrogen fertilizer application in agriculture and the incident light are the main factors determining the nitrate content in plant tissues [8]. Food safety of leafy vegetables (i.e. pak choi) with respect to high nitrate contents is a regular issue in controlled environment horticulture and its newest form, plant factories specially because of over illumination with artificial lights [7,9]. However, the information on the influence of wave length bands (colours) of LED on the nitrate content and other quality parameters of leafy vegetables are incomplete, even though their effects on the crop growth and yield parameters, particularly with respect to the influence of different ratios of red to far-red light (Gilbert et al., 1995; [10,11]) and different combinations of red, blue and green bands of the spectrum [12,13].

Based on these facts, this research examined the comparative advantages of selected plant growing systems for plant growth and leaf yield of leafy vegetables and the effect of different combination of spectral bands of LED on the yield and nitrate accumulation in basil and pak choi.

2. METHODOLOGY

Both experiments were conducted in the AiGROW Green Houses in Colombo, Sri Lanka.

The chemical analyses were done at the Faculty of Agriculture, University of Peradeniya, Sri Lanka during the period of October 2018 to December 2018.

2.1 Experiment 1: Effect of Growing Systems on Greenhouse Grown Basil under LED Lighting

The experiment was conducted as a pot experiment under fully automated greenhouse conditions in a completely randomized design (CRD) with three treatments and six replicates. The treatments were; Top soil (Control; T1), As Compost and coir dust mixture at the rate of 1:1 (T2) and NFT (T3). There were 24 seedlings per each treatment, kept at the spacing of 15 × 25 cm. The pots were made of 300 µ black polythene, having the dimension of 17 × 14 cm, keeping 6-8 punctures for drainage at the bottom. Similar spacing was given for the NFT grown plants where the dimensions of the gully were 127 × 120 cm (length × width). Soil and other media used as the substrate were sterilized using metham sodium before pot filling. Nursery grown basil, variety (Sweet Basil, Sri Lanka) was transplanted to pots after 14 days of nursery period. The plant nutrition was maintained by applying a hydroponics fertilizer, Albert's (CIC, Colombo) in the aqueous phase for all the treatments, except for the control. The pH and EC of the supply solution were 5.9 (at 27.9°C) and 1.5 $mS\cdot cm^{-1}$, respectively. The vegetative growth was evaluated in terms of plant height and leaf/ branch number weekly, starting from four weeks after transplanting (WAT). Dry weights of different plant parts were measured at the harvesting stage (7 WAP) by oven drying the samples (for 48 hours at 70°C). Data analysis was done using software, SAS (Statistical Analysis System) following the Analysis of Variance (ANOVA) and Least Significant Difference test at $P\leq 0.05$ procedures. Non parametric data were analyzed with Chi Square test [14].

2.2 Experiment 2: Effect of Different Led Lighting Solutions on the Quality of Pak Choi

The experiment was a follow up of Experiment 1 and was laid out in the same experimental setup as a CRD with five treatments and 3 replicates, keeping a plot size of 10 plants (Table 1). The growing system was NFT, where channels were arranged in vertically (in multi-shelf racks). Mutual shading due to the shadow of upper racks was compensated by employing LED bulbs at a height of 30 cm above the top of the NFT channel. Plants were placed at a planting density of 225 cm^2, (spacing of 15×15 cm). Supplementary lighting was commenced from 3rd week after seeding (WAS) and continued up to late harvesting period. Leaf samples were analyzed for chlorophyll content at 4 WAS by grinding 0.5g of samples, and keeping the aqueous phase of 80% acetone, following the method of Porra et al. (1989). The nitrate content was determined, following the method of (Catald et al. 1975) where 0.5 g of leaf petiole and leaf blade samples were ground and an aliquot of 0.25 ml of sample extracts were diluted by adding 10 ml and 25 ml of distil water, respectively. Each solution was mixed with 8 ml of 5% salicylic acid in conc. H_2SO_4. After 20 minutes of incubation (at room temperature), 19 ml of 2 N NaOH was added to elevate the pH above 12. When samples were cooled down to

Table 1. Lighting treatments

Treatment	Light source type	PAR value	No of lights/plot	Wattage (w/light)	Intensity (w/plant)	Blue to Red ratio
T1	Horticultural grade LEDs with 1:9 Blue to Red ratio	210-220 ppfd	56	19.8W	110.1	1:9
T2	Horticultural grade LEDs with 1:2 Blue to Red ratio	210-220 ppfd	16	21W	33.6	1:2
T3	Normal LEDs with full spectrum	210-220 ppfd	33	33W	108.9	Full spectrum
T4	Normal LEDs with full spectrum	210-220 ppfd	32	19.2 W	61.4	Full Spectrum
T5	Sunlight	2000 – 2400 ppfd	-	-	27	Full Spectrum

room temperature, spectrophotometer readings were taken at 410 nm for nitrate. Data were analyzed using statistical Analysis System (SAS package). All parametric data were analyzed using analysis of variance (ANOVA) procedure and mean separation was done using LSD method. All nonparametric data were analyzed using Friedman test. Following treatments were used in this study.

3. RESULTS AND DISCUSSION

3.1 Experiment 1: Effect of Plant Growing Systems

In the experiment 01, the growth rate of basil was evaluated in terms of plant height, number of branches, leaves and dry weight of different plant parts (leaves, stems and roots). The results showed that numbers of branches per plant and dry weight of stems were not significantly different among growing systems. Described below are the growth and parameters that are significantly different among different growing systems.

3.1.1 Marketable yield

NFT grown basil showed a significant advancement in marketable yield and dry weight of leaves over the other growing systems (Fig. 1). However, the difference in marketable yield between Soil grown and Compost-coir medium grown basil was not significant. The highest per plant marketable yield (in NFT) was 248.2±12.7 g, while the lowest (in Soil) was 155.5±19.5 g (Fig.1). The results are in agreement with Chandra et al. [15] where average crop yield of basil was 19% higher in aeroponics, compared to those grown in the field. Omaye and Treftz [16] have also reported a 17% higher yield of hydroponically grown strawberries, compared to soil cultivation. Surendran et al. [17] found that the per cent yield as well as quality (i.e. bioactive properties) of hydroponically grown *Mentha spicata* was 61% higher than the conventional soil farming.

3.1.2 Vegetative growth

At the early vegetative growth stage (1-3 WAT), soil cultivation resulted a higher leaf number than the NFT system. However, the rate of leaf emergence was not significantly different during the late vegetative growth (4-6 WAT). However, the highest number of leaves was found in NFT grown basil plants (159 leaves per plant) at harvesting (7 WAT) (Fig. 2).

Meanwhile, the leaf dry weight and root dry weight of basil plants were significantly higher in NFT system than the other growing systems at harvesting (Fig. 3) while there was no significant difference between soil grown and compost grown basil plants. As evident in the reports of many previous studies, hydroponically grown maize (*Zea mays* L.), strawberries, raspberries, and *Mentha spicata* have recorded a greater dry matter accumulation in hydroponics, compared to conventional soil culture under the same planting density (Miller et al. 1989; [16,17]). Meanwhile the results on higher root dry matter accumulation in the NFT system agrees with Miller et al. 1989. To maintain a greater dry matter accumulation, there might be a huge demand for plant nutrients by NFT grown basil plants, resulting in a well developed root system to fulfill this plant nutrient demand. Further, the

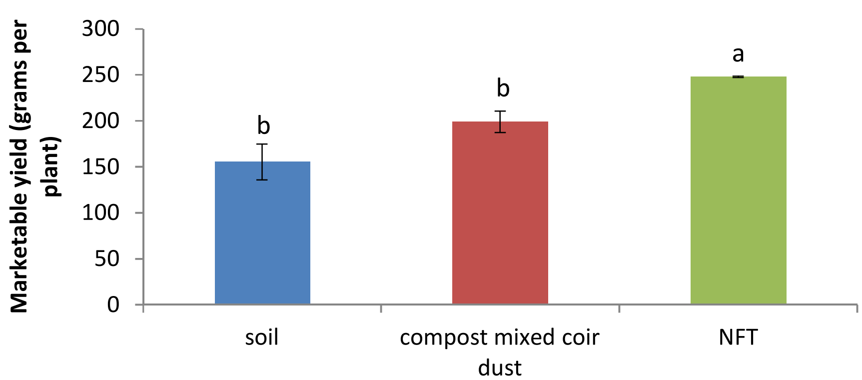

Fig.1. Marketable yield of basil grown in different growing systems
[*Vertical bars indicate the standard error of mean (SEM). Means with different letters are significantly different at p≤ 0.05; LSD = 45.5*]

soil used for growing basil (soil cultivation) contained a higher proportion of clay particles. Therefore, lack of aeration (macro pores) could have been affected the formation and subsequent growth of roots. As a result, the soil grown basil plants have been ended up with the lowest root dry weight. This is well supported by the observations made on the emergence of many tiny roots in both NFT and compost mixed coir dust treatments (data not presented) in the experiment.

As reported by Rouphael et al. [18] zucchini grown in a soilless culture resulted a higher total and marketable yields, fruit numbers, harvest index and water-use efficiency, compared to soil grown plants. Further plants grown in soilless culture contained more N, Mg, Na, Fe, Cu, Mn, and Zn in tissues than those grown in soil. The differences were justified with their less energy requirement for absorbing water in hydroponics and lower risk of oxygen deficiency, compared to soil culture. Some other authors have reported that higher head diameter, number of leaves, head length and dry and fresh total weights of lettuce could be found in hydroponic systems, demonstrating their relative efficiency in crop production the conventional and organic systems mainly due to better plant nutrition offered. According to them, lettuce production in NFT and floating root systems allows harvesting lettuces with weights between 150 and 250 g per head [19-21]. It's a well known fact that maintenance of plant nutrient concentration to fulfill the plant nutrient requirements is one of the most important aspects for successful crop production. Accordingly, precise control of plant nutrition in soilless culture could be the main reason behind the relative yield increment of leafy vegetables in hydroponics [22]. In another study, Selma et al., [23] found that longer growing period of lettuce (102 days) was needed when grown in soil during winter to attain the same level of maturity attained by soilless systems (within 63 days).

3.2 Experiment 2: Effect of Different LED Lighting Solutions

3.2.1 Fresh yield and dry matter accumulation

Supplementary lighting with either horticulture grade or normal LEDs (T1 – T4) significantly increased the leaf fresh weight or the leaf yield, compared to sunlight; control (T5) (Fig. 4). Supplementary lighting with different combinations of red and blue (T1 and T2) could be identified to be better for leaf fresh weight gain compared to full spectrum (T3 and T4)). Although there was a higher fresh weight in T1 (blue and red, at the ratio of 1:9), compared to T2 (blue and red, at the ratio of 1:2), the difference was not statistically significant. The apparently higher fresh weight in T1 could be partly attributed to its higher light intensity. Hence the pak choi yield received under different ratios of blue:red in supplementary LED lighting do not agree with some of the earlier reports on the positive influence of red lights on photosynthesis [24] and plant growth of non-heading type (pak choi) and heading type of Chinese cabbage [25,26] and other leafy vegetables like lettuce [27]. In another experiment, Sase et al., [28] found that shoot fresh weight of lettuce was 22% greater under blue light while 38% greater under red light, respectively than the control (sunlight) without overnight lighting.

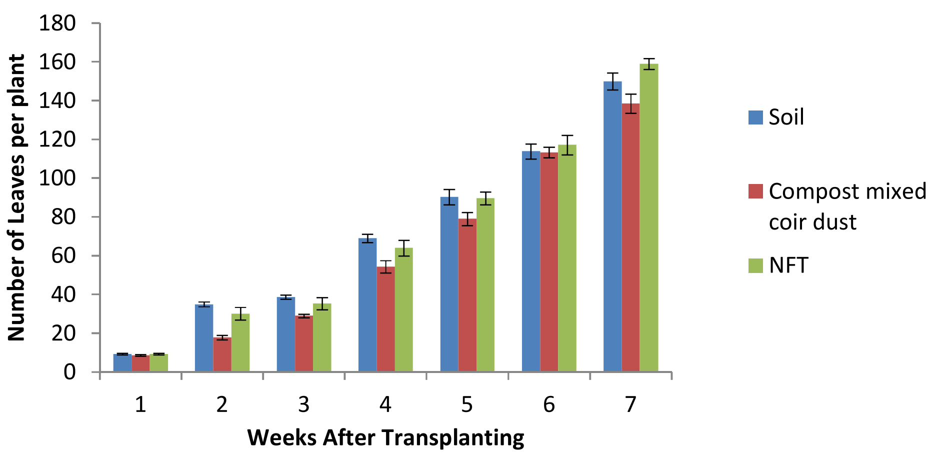

Fig. 2. Number of leaves of basil grown in different growing systems at different growth stages
[*Vertical bars indicate the standard error of mean (SEM)*]

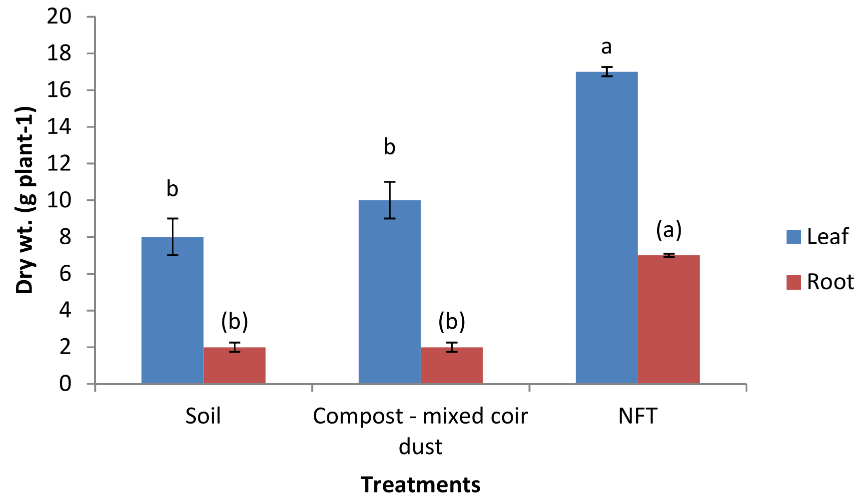

Fig. 3. Leaf dry weight and root dry weight of basil grown in different growing systems
[*Means with different letters are significantly different at p≤ 0.05at LSD = 3.48 for leaf, LSD = 2.35 for root*]

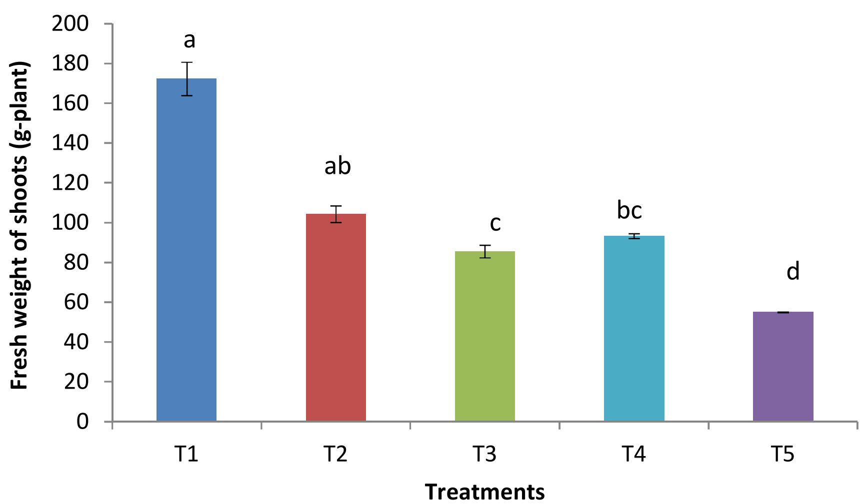

Fig. 4. Shoot fresh weight of pak choi
[*Vertical bars indicate the standard error of mean (SEM). Means with different letters are significantly different at p≤ 0.05*]

According Fig. 5, supplementary lighting with either horticulture grade LEDs with 1:9 Blue to Red ratio (T1) significantly increased the leaf dry weight compared to sunlight; control (T5). Meanwhile, Chen et al. [24] was found that red light is more contributive to photosynthesis and thus to dry matter accumulation and subsequent partitioning to sink organs. Hence the apparently high dry weight of pak choi under T1 could also be due to light quality. It is also supported by few more earlier reports which assures the contribution of red LEDs to dry matter accumulation in non- heading type Chinese cabbage (i.e. Pak choi) [26] and to fresh and dry weights and leaf area of lettuce (var. Sunmang) [29] Meanwhile, blue LED light is important for leaf expansion of leafy vegetables, as reflected by LAI and biomass production [30-32]. Yorio et al. [33] also reported that there was a higher dry matter weight accumulation in lettuce grown under red light supplemented with blue light than red light alone. Di et al. [34] also reported that the changes in photon flux density (R/B ratio) by changing red/blue light ratio could significantly influence the plant growth and development of few leafy vegetables. However, the most appropriate light sources for the best result in dry biomass accumulation are variable with cultivars and species.

3.2.2 Nitrate accumulation in leaves and petiole

Light and nitrogen fertilization have been identified as the major factors that influence nitrate accumulation in vegetables [8]. Influence of supplementary lighting on nitrate accumulation in pak choi leaves was statistically significant at 4 WAP (Fig. 6). Plants in T3 and T4 (LED with full spectrum (61.4-108.9 w/plant) accumulated the lowest nitrates in leaves. Meanwhile T2 (33.6 w/plant), could keep a moderate a nitrate level in leaves similar to sunlight (T5). Significantly lower nitrogen in T2 (33.6 w/plant), compared to T1 revealed the advantage of maintaining low red:blue radiation for maintaining low nitrate contents in pak choi leaves. Hence, high red:blue ratio or its high light intensity could be the reason for high level of nitrate accumulation in the leaves of T1. The latter cause has been reported earlier by Lin et al. [13] for butter head lettuce as nitrate concentration has increased by 33% at the PPFD of 210 µmol m^{-2} s^{-1} under 16h photoperiod.

Irrespective of the source, quality and intensity of light, nitrate contents in the petioles of pak choi were almost twice that of leaves, agreeing with Chen et al. [24] reported for a series of leafy vegetables. Influence of supplementary lighting on nitrate accumulation in the petioles was significant, and the treatment differences were almost similar to the pattern observed for leaf nitrates, except for T3. Unlike the case of leaves, the response of petioles to different ratios of red:blue (difference between T1 and T2) was not significant (Fig. 6), deviating with the results reported on the response of lettuce to supplementary lighting by Urbonaviciut et al. [35] where foliage nitrate accumulation was found lower under high red: blue ratio (86:14).Similar to leaves, a fairly low petiole nitrate contents could be found in T4 and T5 probably because of either low or variable light intensity maintained in them (Fig. 6). The theory of more nitrate accumulation in the dark periods supports the above evidence. And also, theoretically, ability of the foliage of many plant species to reduce their nitrate deposits in foliage when exposed to continuous light for a period (e.g., 2–3 days) before harvest supports this fact.

Further, both leaf and petiole nitrate contents found in this research were below the European Commission's "harmonized maximum level of nitrate contents" for leafy vegetables (3.7 mg/kg body weight per day which is equivalent to 222 mg of NO_3^- per day for a 60 kg individual) [36,37]. Therefore differences in spectral qualities and light intensities in any of the lighting solutions of this experiment has increased leaf or petiole nitrate contents beyond health safety

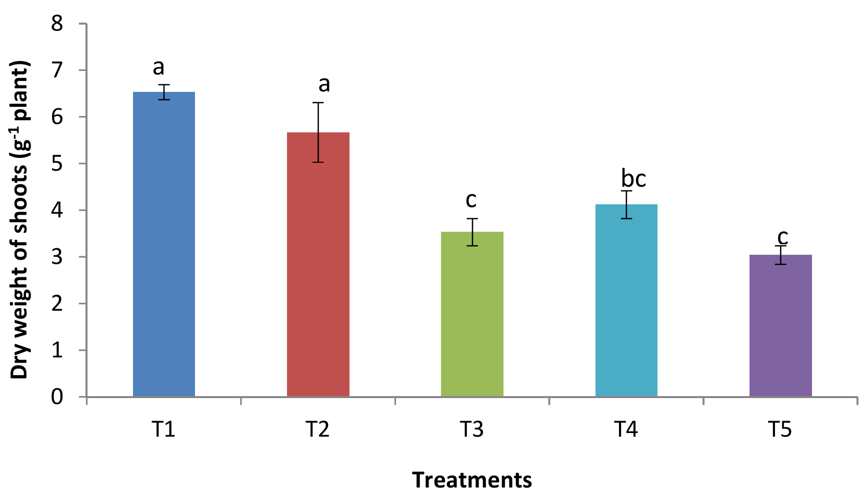

Fig. 5. Shoot dry weight of pak choi

[*Vertical bars indicate the standard error of mean (SEM). Means with different letters are significantly different at p≤ 0.05*]

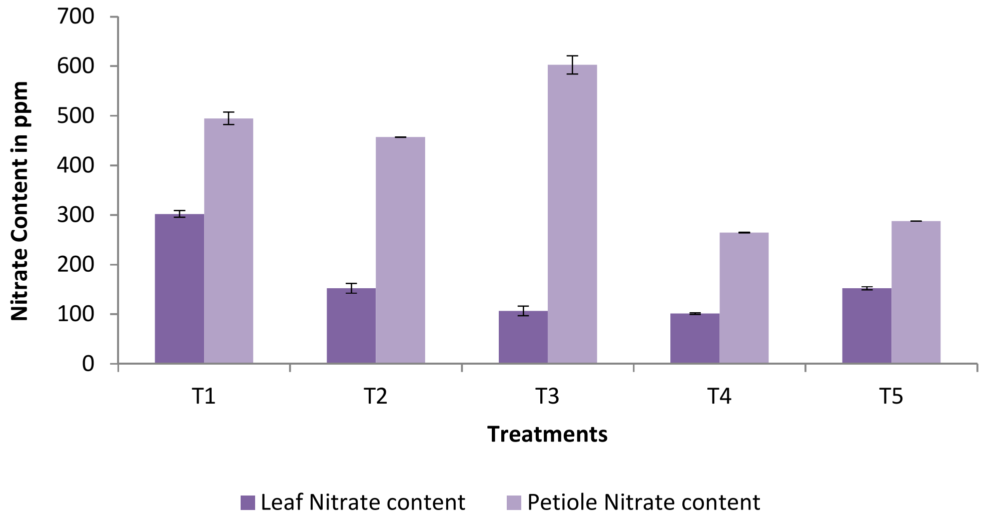

Fig. 6. Influence of supplementary lighting on nitrate accumulation in leaves and petiole
[*Vertical bars indicate the standard error of mean (SEM)*]

margins in pak choi. According to literature, although NO_3^- accumulation could be have been reduced in lettuce by exposing plants to optimal light combinations of red and blue [35] or red, blue, and white [13] or green, blue, and red [9] LED lamps, the blue and red LED light combinations applied in this experiment could not reduce leaf or petiole NO_3^- in NFT grown pak choi.

3.2.3 Total chlorophyll content

The effect of spectral regulation was not significant on total chlorophyll accumulation in pak choi leaves at 4 WAP (at $P \le 0.05$). Generally a range of fresh leaves contain 278 - 310 mg of chlorophyll per100 g fresh weight [38]. The range found in this research was 632 – 2056 mg 100 g^{-1} for pak choi. Total chlorophyll content in leaves increased with time in each spectral treatment in a more or less steady manner but the rate of increase was not significantly different among the treatments. However, Craver et al. [39] found that high red: blue ratio (87:13) in LED light increased total integrated chlorophyll of kohlrabi and mustard.

3.2.4 Chlorophyll a and b contents

Even though chlorophyll a and b contents were determined in the leaf samples of pak choi weekly, the treatment differences were not significant while the rate of increase appeared to be similar to the total chlorophyll contents reported above. The mean chlorophyll a and b ranged within the range of supplementary light treatments within 429 – 1608 and 203 – 569 mg 100 g^{-1}, respectively. Even though some authors have reported the positive influence of blue spectrum on the pigment accumulation in other leafy vegetables, the chlorophyll contents in pak choi did not support this fact [7]. Meanwhile, blue light increased the index of chlorophyll and flavonols as it has been reported to promote the formation of chlorophyll [40] as well as to increase secondary metabolites (Li and Kubota, 2009; [41]). Notably, blue and red LEDs are commonly used for the promotion of plant growth as chlorophyll a and b efficiently absorb blue (absorbance maxima of 430 and 663 nm) and red (absorbance maxima of 453 and 642 nm) wavelengths, respectively [42].

Leaf color is one of the leading quality parameters of leafy vegetables. However, the characteristic leaf color of pak choi was not very dark in nature [43]. Therefore moderate levels of chlorophyll content can be considered adequate to maintain its leaf color [44,45]. Supplementary lighting using horticultural grade LEDs maintained almost similar chlorophyll contents (responsible for green color) to the sunlight as well as the full spectrum LEDs used in the experiment.

4. CONCLUSIONS

NFT grown basil (*Ocimum basilicum* L.) showed a higher shoot and root growth, compared to media (compost-cocopeat mix) based hydroponics and soil cultivation. The resultant marketable yield of NFT system was also higher than the other two systems. Therefore, NFT system can be recommended for basil cultivation for getting a higher vegetative growth and marketable yield, especially under vertical farming.

Leaf and petiole growth of pak choi was exceptionally higher when horticulture grade LEDs with red and blue spectrum were used as a supplementary lighting solution in vertical farming. Even though there is a variation in nitrate accumulation in leaves and petioles of pak choi under different supplementary lighting solutions, all of them kept well below the maximum permissible limits. The influence of supplementary lighting did not influence the chlorophyll contents of pak choi, when compared to sunlight.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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