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Increased Light Intensity and Diluted NPK solution Significantly Influence Lettuce Nitrate Content

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Abstract: This study addressed the issue of quality control which is appealing considerable attention currently to nontoxic and healthy production for human consumption. Excess uptake of nitrate in leafy vegetable is suspected to have carcinogenic effects when consumed in high quantity in a human diet. In this regard, we investigated a strategy to minimize nitrate content in close biological production system a few days before harvest. We evaluated the proposed innovate scheme through Fluorescent, LED and hybrid lamps. at different PPF levels. During growth, environmental factors were monitored by the environmental control system. Japanese Yamazaki nutrient solution NPK100 delivered to all treatments between EC 1.0 to 1.2 mScm^{-1} and PH 6.0 ~ 6.5 to gain average target weight, three days before harvest treatments were subdivided into different sections containing different concentration levels of nitrate in solution as N100/N50/N0/N50L, The N50L experimental zone received additional PPF from 250µmolm^{-2}s^{-1} to 300µmolm^{-2}s^{-1} from rest of treatments. Our data appeared to suggest that plant treated with N50% with additional light intensity witnessed fall in leaf nitrate values accordance with a critical level set by European standard, most decreased leaf nitrate content found with the fluorescent lamp under treatment section FLP300-NPK50L, fresh and dry weight exhibited higher in same section. This work has highlighted and revealed a marked striking effect on quality control and limiting nitrate concentration in lettuce indoor production facilities without losing yield.

Keywords: Light quality, intensity, nitrate content, solution conductivity.

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1. Introduction

Leafy vegetables are important constituent of human diet due to their health-giving benefits that inhibit cardiovascular diseases and reduce the risk to cancer (Dias, 2019; Nyathi et al., 2018; Weiguo et al., 2012). Due to quality deterioration of product on the commercial scale, interest of consumers and policymakers of indoor production gradually risen in improving the quality of leafy vegetables with low nitrate content which is beneficial for human consumption (Ding et al., 2018; Carlström et al., 2018; Jackson et al., 2018; Lundberg et al., 2018; Wang et al., 2012). Lettuce (*Lactuca sativa* L) is commonly consumed as a fresh salad in sandwiches with high nutritional value (Boriss and Brunke, 2005; Franz, 2018; Spence, 2020; Wojicechowska et al 2015). Lettuce is of significant agricultural and economic importance.

However, higher nitrate concentration when grown in greenhouse and plant factory is of great health concern (Bian et al., 2018; Darko et al 2014; Zandvakili et al., 2018; Zandvakili et al., 2019; Zhou et al., 2012). Due to quality deterioration of product on the commercial scale, interest of consumers and

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policymakers of indoor production gradually risen in improving the quality of leafy vegetables with low nitrate content which is beneficial for human consumption. (Wang et al 2012). Lettuce cultivation in close plant factory system requires precise management of light quality, intensity balanced solution composition for successful economic yield (Bian et al 2015). Subsequently, fluctuations in abiotic growth factors in a close hydroponic system potentially cause nutrient imbalance with high precision accumulation of nitrate in aerial parts of leafy green vegetables (Liu et al 2012; Loconsole et al., 2019). Considerable variations have been reported in nitrate concentration level in hydroponically grown lettuce as compared to conventional land cultivation (Lastra O et al., 2009; Schuerger et al. 1997; Seifu YW et al 2017).

Light and nitrogen play a significant role in plant growth and development (Ata-Ul-Karim et al., 2017; Nasim et al., 2018). As Plants accumulates more nitrate under low light conditions, due to inhibition of nitrate reductase activity (Ohse, et al 2000; Kaiser et al., 2011). Moreover, plant tissues accumulate more nitrates when uptake of nitrogen exceeds than requirement as in case of higher nitrogen fertilization. (van der Leij et al.,1998 Liu et al. 2004, Tamulaitis et al. 2005). Previous studies have shown that light quality, intensity and concentration of nitrogen in the hydroponic solution regulates and induces the main enzyme activity of metabolism of nitrate morphological characters as well as anti-oxidative activities during growth (Fu et al 2012, Darko et al 2014, Bian et al., 2015).

Short term pre-harvest and temporal changes during growth affect nitrate accumulation, spectral distribution of growth irradiance and solution concentration potentially involved in nitrate uptake from root zone environment to leaves tissues. Withdrawal of the nitrogen availability in growth medium, cutting nitrogen supply in nutrient solution to half before harvest accommodates plant tissues to reduces nitrate content significantly (McCall and Willumsen, 1999; Santamaria et al 2001; saigusa and Kumazaki 2014).Subsequently Light quality has been one of the most important factors which influence almost every physiological process of plants, not only photosynthesis, but also metabolism. Investigation by (McCall and Willumsen, 1999,Samuoliene et al. 2009) showed that using Red LED could considerably reduce the nitrate content in lettuce leaves, or an increase in Blue light intensity also increased phenolic contents and antioxidant capacity of red leaf lettuce (Johkan et al.2010; Son and Oh 2015). Nutritional values of lettuce can be strategically controlled by supplementing blue and red light to activate the biochemical enzymes secondary metabolite (Jenkins 1997, Son et al, 2012; Lin et al., 2013; Taulavuori et al., 2016).

This study was to conducted to determine effect of light quality, light intensity and nitrate solution concentrations on morphological attributes and quality of lettuce. Our current work objective was to develop a quality control strategy in edible parts of lettuce that cultivated in greenhouses and plant factories. This study presents new insights into the combinational regulation of light intensity and nitrogen supply to improve quality and production to minimize and optimize nitrate concentration so that results could be conducive for public health and policy makers of indoor production system.

2. Materials and Methods

2.1. Plant Material and Experimental Procedure

Lettuce type Japanese wrinkled leaf (*cv Frill ice*) chosen as plant material and cultured hydroponically in plant factory of China Agricultural University, Beijing, China, under the ambient artificial light condition of growth. Planting board balanced on fustigationbed each complete board planted 24 lettuce plants. Transplanted plants were exposed different PPF level of 150 µmolm-^{2}s-^{1} and 250 µmolm-^{2}s-^{1} in environmental control system (Table 1) with photoperiod of 16hd-^{1,} temperature 22°C/18°C (L/D), CO_2 concentration: 800±50µmolmol-^{1}, Relative humidity(70±5%). The R: B ratio of lamps were as follows Fluorescent Lamps (R: B=2) LED (R: B=1) Hybrid Lamps (R: B=1.5) respectively.

2.2. Nutrition Management

The elemental composition of standard hydroponic solution in mgL^{-1} (NPK100/NPK50) were Ca $(NO_3)_2 \cdot 4H_2O$, 236/123, KNO_3 404/202, $MgSO_4 \cdot 7H2O$ 123/123, NH_4H2PO_4,57/28.5. The concentration of microelements mgL^{-1} were Na_2Fe-EDTA 30, H_3BO_3 2.86, $MnSO_4 \cdot 4H_2O$ 2.13, $ZnSO_4 \cdot 7H_2O$ 0.22, $CuSO_4 \cdot 5H_2O$ 0.08, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.02). Three days before harvest. Solution subdivided into four section NPK100/NPK50/NPK0/NPK50L. The NPK50 sections adjusted with $Cacl_2$ (55.5 mgL^{-}) and NPK0 only with micronutrient to avoid appearance of abnormal growth deficiency in absence of N in solution. PH of 6.0 ~ 6.5, and EC 1.0 to 1.2 mS cm-^{1} of solution were maintained till harvest.

Table 1. Experimental treatments with different light qualities, PPF and Nitrate solution conductivity

Light Source	PPF (μmol $m^{-2}s^{-1}$)	Solution concentration	Name of section
Fluorescent lamp (R: B=1.9)	150	N100/P100/K100	F150-NPK100
		N50/P50/K50	F150-NPK50
		N0/P0/K0	F150-NPK0
		N50/P50/K50+P300	F150-NPK50L
	250	N100/P100/K100	F250-NPK100
		N50/P50/K50	F250-NPK50
		N0/P0/K0	F250-NPK0
		N50/P50/K50+P300	F250-NPK50L
LED lamp (R: B=1.3)	150	N100/P100/K100	L150-NPK100
		N50/P50/K50	L150-NPK50
		N0/P0/K0	L150-NPK0
		N50/P50/K50+P300	L150-NPK50L
	250	N100/P100/K100	L250-NPK100
		N50/P50/K50	L250-NPK50
		N0/P0/K0	L250-NPK0
		N50/P50/K50+P300	L250-NPK50L
Hybrid lamp (R: B=1.6)	150	N100/P100/K100	H150-NPK100
		N50/P50/K50	H150-NPK50
		N0/P0/K0	H150-NPK0
		N50/P50/K50+P300	H150-NPK50L
	250	N100/P100/K100	H250-NPK100
		N50/P50/K50	H250-NPK50
		N0/P0/K0	H250-NPK0
		N50/P50/K50+P300	H250-NPK50L

2.3. Growth Analysis

Lettuce morphological attributes analyzed in each experimental zone on day 0, 20, 23. After recording fresh weights, shoot and roots were oven dried at 90℃ ± 15℃, for 72 h.

2.4. Nitrate Contents

The identification of leaf nitrate concentration carried out by reliable sulfosalicylic acid method, In the first step Sampled fresh leaves were cut into pieces, mixed, weighted (2-3 g) on electronic balance and placed in graduated test tube of 10 ml filled with deionized water, the tubes then sealed with plastic wrap, placed in boiling water for 30 mints to extraction , the extract filtered into 50 ml flask with distilled water up to the mark, for sample test of leaf nitrate about 0.1 ml from extracted solution taken in graduated test tube, further added 0.4 ml (5% salicylic acid - sulfuric acid) solution at room temperature left for 20 min, then carefully added 9.5 ml 8% NaOH solution, after cooling to room temperature samples passed at wavelength of 410nm by using spectrophotometer.(UV-VIS-NIR Scanning Spectrophotometer /UV-3150 Shimadzu Japan) for identification of nitrate level.

2.5. Lettuce Sugar Content

Sugar contents (%) in lettuce leaves were determined following procedure as describe in Anthronecolorimetry method. Samples were exposed to absorbance level of 630 nm wavelength by using instrument (UV-VIS-NIR Scanning Spectrophotometer /UV-3150 Shimadzu Japan).

2.6. Chlorophyll Content

Chlorophyll pigments determination carried out by explained producers in accordance with 80% acetone concentration at absorbance levels of 663 nm and 645 nm using (UV-VIS-NIR Scanning Spectrophotometer /UV-3150 Shimadzu Japan). Aron’s (1949) equations used for further calculation.

2.7. Photosynthesis

The photosynthetic rate, transpiration rate, intercellular CO_2 concentration, and stomatal conductance, were measured by using portable photosynthesis system (LI-6400, LI-COR Biosciences, Lincoln, NE, USA).

2.8. Statistical analysis

The experimental data were carefully obtained and analyzed by using Microsoft Excel 2010 application, and SPSS program. The data subjected to ANOVA at the 0.05 significance level using LSD and Duncan method for multiple comparisons test between different sections marked * Indicated a significant difference, marked NS indicates no significant difference between experimental zones.

3. Results and Discussion

Increasing light intensity in combination with decreasing the N availability in solution had strong influence and decreased the nitrate content in lettuce leaves. Morover, light quality, intensity significantly affected the morphological attributes and quality of lettuce which indirectly reflected plant growth status and results were substantial on growth morphology, photosynthesis and chlorophyll content. Furthermore, nitrate uptake in hydroponic system responded to light intensity and quality dependently that usually works under variety of ways with solution strength (Table 2). Light intensity generally drives the energy for metabolism regulating mechanism of utuilization of light quality which accelerate photosynthetic activity (Folta and Childers, 2008).

The nitrate content found in leaves of NPK100 experimental zone were higher but it reduced gradually and reached to minimum in treatment section P300NPK50L, where as in contrast we found increased soluble sugar content in same experimental zone. Lettuce response to accumulation of leaf nitrate found in significant difference due to light quality, intensity and its response to N concentration in solution. Treatments with Low light intensity accumulates more nitrate and that point coincides with substantial decrease in nitrate with high PPF. Current results agreed with previous findings reporting negative correlation existed between nitrate concentrations with high light intensity (Boon et al., 1990; He, 2010; McCall and Willumsen, 1999).

Table 2. Effect of different Lighting environment and nutrient solution strength (NPK100) on nitrate content of hydroponic lettuce at day 0, 20 and effect of nitrate solution treatment before harvest at day 23.

Sections		Day 0				Day 20				Day 23			
FL-150	P150NPK100	0.84	±	0.42	NS	6.07	±	1.78	b	7.5	±	0.51	ab
	P150NPK50	0.84	±	0.42	NS	6.07	±	1.78	b	6.55	±	1.8	bc
	P150NPK0	0.84	±	0.42	NS	6.07	±	1.78	b	3.94	±	1.44	cd
	150NPK50L	0.84	±	0.42	NS	6.07	±	1.78	b	4.16	±	0.4	cd
FL-250	P250NPK100	0.84	±	0.42	NS	5.47	±	0.84	b	6.59	±	0.99	b
	P250NPK50	0.84	±	0.42	NS	5.47	±	0.84	b	4.52	±	0.31	cd
	P250NPK0	0.84	±	0.42	NS	5.47	±	0.84	b	2.61	±	0.29	d
	P250NPK50L	0.84	±	0.42	NS	5.47	±	0.84	b	2.25	±	0.16	d
LED-150	P150NPK100	0.84	±	0.42	NS	7.21	±	1.54	ab	8.07	±	0.74	a
	P150NPK50	0.84	±	0.42	NS	7.21	±	1.54	ab	4.16	±	0.17	d
	P150NPK0	0.84	±	0.42	NS	7.21	±	1.54	ab	2.52	±	0.14	d
	P150NPK50L	0.84	±	0.42	NS	7.21	±	1.54	ab	5.49	±	0.53	bc
LED-250	P250NPK100	0.84	±	0.42	NS	4.79	±	0.46	b	6.09	±	0.23	bc
	P250NPK50	0.84	±	0.42	NS	4.79	±	0.46	b	4.09	±	0.73	cd
	P250NPK0	0.84	±	0.42	NS	4.79	±	0.46	b	2.72	±	0.53	d
	P250NPK50L	0.84	±	0.42	NS	4.79	±	0.46	b	3.7	±	1.59	cd
HYBRID-150	P150NPK100	0.84	±	0.42	NS	8	±	2.55	a	8.03	±	1.23	a
	P150NPK50	0.84	±	0.42	NS	8	±	2.55	a	5.25	±	0.76	c
	P150NPK0	0.84	±	0.42	NS	8	±	2.55	a	2.52	±	0.26	d
	P150NPK50L	0.84	±	0.42	NS	8	±	2.55	a	4.56	±	0.9	cd
HYBRID-250	P250NPK100	0.84	±	0.42	NS	5.73	±	0.77	b	6.14	±	0.63	bc
	P250NPK50	0.84	±	0.42	NS	5.73	±	0.77	b	3.23	±	0.48	bc
	P250NPK0	0.84	±	0.42	NS	5.73	±	0.77	b	2.39	±	0.35	d
	P250NPK50L	0.84	±	0.42	NS	5.73	±	0.77	b	4.72	±	0.37	cd

Table 1. Effect of Light quality, intensity and Nitrate solution concentration on FW, DW and quality of hydroponic lettuce at day 23 after Transplanting

Sections		FSW g/plant	DSW (g/plant)	Sugar (%)	Phosphorus (mg/plant)	Potassium (mg/plant)
	P150NPK100	83.6 ± 0.68 cd	3.7 ± 0.62 bc	3.4 ± 0.2 b	3.4 ± 0.5 c	1.22 ± 0.7 ab
FL-150	P150NPK50	80.7 ± 0.42 cd	3.7 ± 0.86 b	3.7 ± 0.4 b	3.1 ± 0.1 cd	1.01 ± 0.4 ab
	P150NPK0	74.1 ± 3.91 d	3.5 ± 0.34 bc	3.6 ± 0.6 b	1.9 ± 0.3 d	1.25 ± 0.1 ab
	150NPK50L	88.2 ± 2.33 b	3.2 ± 0.09 bc	3.4 ± 0.3 b	2.4 ± 0.1 d	1.31 ± 0.9 a
	P250NPK100	102 ± 3.14 ab	4.3 ± 0.92 ab	4.4 ± 1 b	3.7 ± 0.2 bc	0.95 ± 0.4 ab
FL-250	P250NPK50	99 ± 5.38 ab	4.6 ± 0.16 ab	2.7 ± 0.6 b	3.7 ± 0.5 bc	0.56 ± 0.1 b
	P250NPK0	97.6 ± 6.47 bc	4.8 ± 0.58 ab	4 ± 1 b	2.9 ± 0.2 cd	0.27 ± 0.1 b
	P250NPK50L	115 ± 4.49 a	4.8 ± 0.81 a	5.2 ± 0.5 ab	4.7 ± 0.4 a	0.77 ± 0.3 ab
	P150NPK100	89.9 ± 0.88 bc	2.8 ± 0.45 c	3.4 ± 0.6 b	3.9 ± 0.3 bc	0.91 ± 0.3 ab
LED-150	P150NPK50	80.4 ± 9.53 cd	3.6 ± 0.71 bc	3.3 ± 1.3 b	3.1 ± 0.5 c	1.07 ± 0.2 ab
	P150NPK0	78.2 ± 4.57 d	3.5 ± 0.18 bc	3.6 ± 0.8 ab	2.2 ± 0.4 d	0.37 ± 0.1 b
	P150NPK50L	90.9 ± 0.43 ab	2.9 ± 0.58 bc	4.3 ± 0.4 b	3.7 ± 0.6 c	0.38 ± 0.2 b
	P250NPK100	100 ± 9.53 ab	3.4 ± 0.15 bc	4.3 ± 0.4 b	3.7 ± 1.2 bc	0.97 ± 1.1 ab
LED-250	P250NPK50	95.5 ± 1.73 bc	3.8 ± 0.18 ab	2.7 ± 1.2 c	3.3 ± 0.6 c	0.54 ± 0.1 b
	P250NPK0	99.5 ± 2.02 bc	3.6 ± 0.91 bc	2.8 ± 0.9 c	2.6 ± 0.1 d	0.65 ± 0.1 b
	P250NPK50L	101 ± 7.35 ab	4.4 ± 0.52 ab	4.2 ± 0.5 b	3.8 ± 1.5 bc	0.3 ± 0.1 b
	P150NPK100	87.7 ± 2.92 bc	3.9 ± 0.71 ab	4.2 ± 0.7 b	3.5 ± 0.6 c	0.71 ± 0.3 ab
HYBRID-150	P150NPK50	81 ± 2.09 cd	2.3 ± 0.3 c	3.9 ± 0.6 b	3.9 ± 0.2 bc	0.22 ± 0.2 b
	P150NPK0	75.3 ± 2.15 d	3.5 ± 0.29 bc	4 ± 0.7 b	2.5 ± 0.3 d	0.46 ± 0.1 b
	P150NPK50L	86.8 ± 0.75 bc	3.5 ± 0.54 bc	3.8 ± 0.3 b	4.3 ± 0.3 a	0.48 ± 0.1 b
	P250NPK100	101 ± 1.59 ab	3.6 ± 0.52 bc	5.5 ± 1.8 a	4 ± 0.2 bc	0.63 ± 0.3 b
HYBRID-250	P250NPK50	97 ± 4.14 bc	4 ± 0.95 ab	4 ± 0.2 b	3 ± 0.6 cd	0.43 ± 0.3 b
	P250NPK0	100 ± 4.25 ab	3.6 ± 0.65 bc	2.4 ± 0.4 c	2.5 ± 0.5 d	0.71 ± 0.6 b
	P250NPK50L	102 ± 2.24 ab	4.1 ± 0.35 ab	5 ± 1.4 ab	4.9 ± 0.4 a	0.36 ± 0.4 b

Our results observed that diluting the nitrogen in solution and providing additional light intensity at pre-harvest stage may fall down movement of nitrate form roots to shoots and stabilize the nitrogen metabolism and accumulation of nitrate in leaves. This indicates that decrease in specific nutrient absorbed in hydroponic system is dependent upon solution conductivity that usually works under variety of ways under light quality and intensity in close plant factory system. Similar results were found by (Wang et al., 2012) that nitrate uptake and its reduction is light dependent. Application of increasing light intensity and decreasing the N in solution strongly effected hydroponic cultured lettuce, and decreased the NO_3^- content in lettuce. Sugar and photosynthetic characteristic of investigated lettuce found higher in Hybrid lamps. This light combination can be beneficial to accelerate higher net photosynthetic rate, high sugar indicates sweeter taste. The lettuce growth under manipulation of fluorescent and LED (Hybrid lamp) shown efficiently working of chlorophyll molecules that favors opening of stomata under and increases the net photosynthesis rate. Plants exposed to such light source definitely be advantageous and beneficial to activate metabolic changes. Our data agreed with finding reported by (Lin et al., 2013).

The observed found pattern of phosphorous and potassium in lettuce leaves to different light qualities, intensities was proportional to solution strength and mineral contents near root zone. These results agreed with previous finding that nutrient uptake by plants depend upon growth requirements as reported by (Hogewoning et al., 2010; Kim et al., 2004; Li and Kubota, 2009). Amoozgar et al., (2017) further light intensity, duration, spectral composition of lights and availability of N in solution cannot be separated on mineral uptakes (Johkan et al., 2010). The results of morphological attributes showed that all the treatment sections exposed to same environmental conditions for growth till day 18. Light quality and intensity and various difference among treatments responsible for higher and lower biomass. Our results agreed with previous reports (Kim et al., 2004; Yorio et al., 2001) indicating better growth response of lettuce under FL as compared to LED. Light quality management played significant role in plant physiological process and nutrient uptake. Plant grown under artificial lights not only impulse to light intensity, spectral composition but also respond to nutrient uptake in solution as reported by (Hogewoning et al., 2010). Fluorescents lamp associated with red and blue and some proportion of green lights in its spectral composition which almost absent or ineffective in LED light which is reported beneficial for plant growth in literature. According to (Klein, 1992) fluorescent lights spectral composition has unique ability to pass thorough the plant canopy.Another study (Sun et al., 1998) also reported increased carbon assimilation in lower leaves due to green light addition to red and blue using fluorescent lights.

Plants exposed to different light intensities which subsequently effects net rate of photosynthesis and limits the cell expansion during growth, that might be possible reason behind growth depression under low PPF 150.Our results findings matched with previous studies that light intensity increases and promotes lettuce growth (Li and Kubota, 2009; Loomis and Amthor, 1999) optimization of light intensity plays a crucial role in growth and development that works well under specific conditions to evoke photosynthetic responses with growth stages. The PPF and R: B ratio in light sources must be in appropriate combination to facilitate quality production before exposing it to plants and to avoid growth depression. The findings results also indicates that absorption ability of given nutrients in medium depends upon light quality ,intensity and specifics concentration of mineral elements in solution to contribute complete growth ,additional PPF after day closely related to higher photosynthetic rate .These findings are consistent to results reported by (Cookson et al., 2005; Riens and Heldt, 1992). we further suggest that additional research is needed to design a modified solution to optimize production and mineral contents and utilization of nutrient use efficiency under different light qualities and intensities in regulation of nitrate content.

4. Conclusion

We conclude that indoor lettuce production with real low nitrate content exists with precise management strategy of combining higher light intensity on lower nitrogen solution before harvest for a safe human consumption without losing yield. It is more important to take in consideration the environmental factors in greenhouses and plant factory with growth requirements of plants. However,quality of light and nitrogen combination needs further investigation for sustainable lettuce production.

List of Abbreviations: CRD: Completely randomize design; CP: Crude protein; P: Pleurotus; S: Substrate; WS: Wheat straw; NP: Newspaper; PN: Pine needles; BE: Biological efficiency; gkg^{-1} : gram per kilogram; ANOVA : Analysis of variance; LSD: least significant difference.

Competing Interest Statement: All the Authors declare that they have no competing interest.

Author's Contribution: Khan, Hussain and Ikram ul Haq conceived and designed of the study. Khan conducted the experiment. Maqbool, Ahmad, Ali and Hameed performed the data analysis. Khan, Ikram ul Haq and Noureen wrote the manuscript. All the authors read and approved the final manuscript.

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