

Effect of the Nitrate-Urea Ratio, Nickel Levels, and Light Intensity on the Development of *Lactuca sativa* L. under Hydroponic Conditions

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ABSTRACT

Objective: To determine the effects of the nitrate/urea ratio, nickel concentrations in the nutrient solution, and different light intensities on the development of lettuce cultivated in a hydroponic system.

Design: The experiment was conducted using a completely randomized design with a $2 \times 3 \times 3$ three-factor arrangement, comprising 18 treatments and five replicates. The evaluated factors were the nitrate/urea ratio, nickel concentration, and light intensity.

Results: The 80/20 nitrate/urea ratio promoted the greatest root volume, leaf fresh weight, leaf and root dry weight, and larger leaf area, thus indicating higher efficiency in nitrogen assimilation when part of the supply was provided as urea. Nickel at a concentration of 1 mg L^{-1} tended to enhance growth compared with the absence of nickel. Likewise, a light intensity of $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$ increased root volume, dry biomass, and leaf greenness.

Study limitations/implications: Further research is needed to evaluate other mineral sources and different specific spectral compositions for production under controlled environments, as these represent a promising opportunity to improve the development and quality of hydroponic lettuce.

Findings/conclusions: Nickel exerted moderately positive effects at a concentration of 1 mg L^{-1} , and its combination with the nitrogen source ratio of 80/20 under a light intensity of $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$ resulted in greater root volume, dry biomass, and leaf greenness (SPAD). Therefore, this treatment can be considered a viable alternative for improving lettuce development in hydroponic systems.

Keywords: lettuce, hydroponics, light, nickel, nitrogen.

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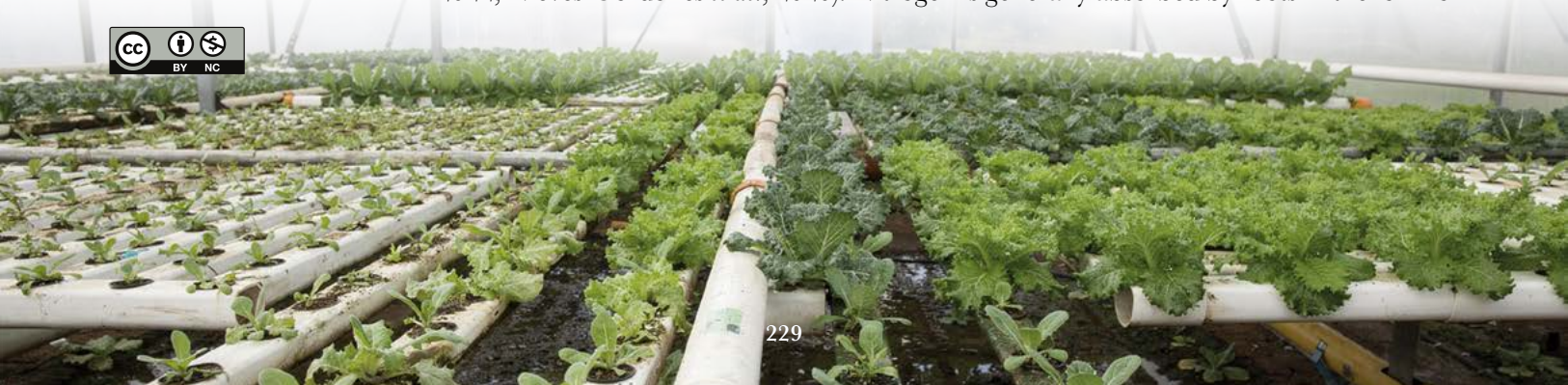
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INTRODUCTION

Nitrogen (N) is the most important and essential element for the development and growth of most plants. It is present in the highest proportion, accounting for 1 to 3% of dry matter, depending on the species, phenological stage, organ, and other factors. It enhances the photosynthetic process and increases leaf area, biomass production, and yield (Fathi, 2022; Nieves-Cordones *et al.*, 2020). Nitrogen is generally absorbed by roots in the form of



nitrate, ammonium, and urea (Buoso *et al.*, 2023). Nitrate (NO_3^-) is preferentially absorbed and metabolized by most plant species, particularly under hydroponic cultivation, where it is reduced to nitrite (NO_2^-) and subsequently to ammonium (NH_4^+), before being incorporated into plant organs as amino acids, chlorophyll, and nucleic acids (Hong *et al.*, 2025). Moreover, nitrate plays an important regulatory role in growth and development processes and in water balance. It is the form most commonly absorbed by most species, especially in hydroponic systems and well-aerated soils, and it promotes a more alkaline rhizosphere pH due to the uptake of accompanying cations (Nieves-Cordones *et al.*, 2020).

Humans consume nearly 80% of dietary nitrate from vegetables (Karwowska *et al.*, 2020). In contrast, nitrate accumulation in most leafy vegetables remains a major concern because it is reduced to nitrites and nitroso compounds, accumulates in cellular vacuoles, and becomes carcinogenic when consumed in large quantities (Karwowska *et al.*, 2020). The ammoniacal form is readily assimilable; however, it must be carefully regulated by the plant, since high concentrations may cause toxicity and are therefore scarcely recommended in vegetables (Dong *et al.*, 2023). Another nitrogen source is urea, a fertilizer of great importance due to its high nitrogen content and low cost, although it is still scarcely used in hydroponic crops. Plants can absorb urea through their roots, after which it is hydrolyzed to ammonium by the enzyme urease, whose activity depends on nickel (Ni) availability (Gupta and Pathak, 2025).

Consequently, nickel supplementation is required to promote more efficient urea hydrolysis in plants (Khan *et al.*, 2000). Nickel is absorbed by plant roots as the Ni^{2+} cation (Brown, 1987; Villegas *et al.*, 2015) and transported to the shoot biomass, from where it may be mobilized and accumulated in leaves, seeds, and fruits in the form of complexes with organic acids (Brown, 2006). The average nickel content in lettuce leaves ranges from 1.0 to 1.8 mg kg^{-1} dry weight (Kabata, 2000; Guzmán *et al.*, 2021). Appropriate light intensity can influence the availability of micronutrients and nitrogen, thereby altering growth and metabolism (Reyes-Rosas *et al.*, 2025). Recent studies have demonstrated that modifying the nitrogen source in the nutrient solution significantly influences nitrogen use efficiency and crop yield. Jurga *et al.* (2021) showed that changes in nitrogen composition alter nitrogen uptake and utilization in hydroponic lettuce, underscoring the relevance of strategies aimed at optimizing its assimilation. Previous studies, such as those reported by Aguilar *et al.* (2025), demonstrated that foliar application of nickel (0.3 g L^{-1}) combined with urea at concentrations of 4 and 8% significantly improved photosynthetic performance, pigment levels, and nitrogen metabolism in cotton plants. In lettuce, the effect of nickel additions has also been evaluated, showing that low concentrations ($0.5\text{-}1.0 \text{ mg L}^{-1}$) significantly improved urease activity and nitrogen utilization when urea was used as the nitrogen source, but had no effect or even reduced growth when nitrate was supplied (Khoshgoftarmanesh *et al.*, 2011).

Rizwan *et al.* (2022) investigated nickel toxicity in rice under different nitrate/ammonium combinations and reported that, at concentrations above 1.0 mg L^{-1} , Ni reduced nitrate and ammonium uptake, affecting nitrate reductase (NR) and glutamine synthetase (GS). Furthermore, Pennisi *et al.* (2020) and He *et al.* (2020) reported that

moderate to high light intensities increased photosynthesis, biomass, and resource use efficiency in leafy vegetables cultivated under light-emitting diode (LED) lighting. Accordingly, lettuce productivity may be positively affected by modulating light intensity (Loconsole *et al.*, 2019). Therefore, the effect of the nitrate/urea ratio, nickel concentrations in the nutrient solution, and different light intensities on the development of lettuce grown in a hydroponic system was evaluated.

MATERIALS AND METHODS

Location of the study area

This research was conducted at the Laboratory of Plant Physiology and Anatomy, Faculty of Agronomy, Autonomous University of Sinaloa, Mexico (24° 37' 29.8" N, 107° 26' 37.7" W), under a warm climate characterized by very hot and humid summers, dry and mild winters, and an annual mean temperature ranging from 24 to 40 °C.

Experimental management

As plant material, romaine lettuce plants (RIJK ZWAAN MAXIMUS RZ) were used. This is a green Cos- or Romaine-type lettuce variety. It is a highly versatile material that can be cultivated throughout the year and, under warm conditions, exhibits high tolerance to bolting and tip burn, making it widely used in hydroponic systems. Sowing was carried out in 200-cell polystyrene trays filled with peat moss (PRO-MIX® FLX, Premier Tech Horticulture, USA). When the plants developed their first five true leaves (30 days after sowing), they were individually transplanted into circular plastic pots measuring 11.5 × 15.5 × 13.5 cm, filled with vermiculite up to the pot surface. The pots were placed in three growth chambers with 1.94 m³ of usable space under controlled temperature conditions (22-24 °C), relative humidity of 60-80%, and ambient carbon dioxide. Lighting was provided by transparent white LED tubes (T8 LED 30 W, 6500 K, 2400 lm; MRGL-08, Megaluz, Mexico). The three growth chambers each contained seven white LED tubes positioned uniformly at different heights according to the intensity delivered at the plant canopy, thereby achieving three light intensities in each chamber: 200, 300, and 400 μmol m⁻² s⁻¹, respectively, with a photoperiod of 15 h per day. Transplanting was performed on June 15, 2025, and the harvest period began 36 days after transplanting.

Preparation of nutrient solutions

The nutrient solutions were prepared using water-soluble inorganic salts dissolved in distilled water. Universal nutrient solutions (Steiner, 1984) were modified to adapt them to the different nitrate/urea proportions (Table 1). The fertilizers used to prepare the nutrient solution were calcium nitrate, potassium nitrate, magnesium sulfate, potassium sulfate, monopotassium phosphate, calcium sulfate, and urea. Micronutrients were added using Fullmix Chelate B as the source, with the following concentrations in the nutrient solutions: iron (Fe), 3.0 mg L⁻¹; manganese (Mn), 1.48 mg L⁻¹; boron (B), 0.28 mg L⁻¹; copper (Cu), 0.12 mg L⁻¹; zinc (Zn), 0.24 mg L⁻¹; and molybdenum (Mo), 0.08 mg L⁻¹.

Table 1. Chemical composition of the nutrient solutions and light intensities used in each treatment of the experiment.

Treatments	PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Nickel (mg L^{-1})	NO_3^- (meq L^{-1})	H_2PO_4^- (meq L^{-1})	SO_4^{2-} (meq L^{-1})	K^+ (meq L^{-1})	Ca^{2+} (meq L^{-1})	Mg^{2+} (meq L^{-1})	$\text{CO}(\text{NH}_2)_2$ (meq L^{-1})
100-0*0*200	200	0	12.0						
100-0*1*200	200	1	12.0	1.0	7.0	7.0	9.0	4.0	0.0
100-0*2*200	200	2	12.0	1.0	7.0	7.0	9.0	4.0	0.0
80-20*0*200	200	0	9.6	1.3	9.1	7.0	9.0	4.0	2.4
80-20*1*200	200	1	9.6	1.3	9.1	7.0	9.0	4.0	2.4
80-20*2*200	200	2	9.6	1.3	9.1	7.0	9.0	4.0	2.4
100-0*0*300	300	0	12.0	1.0	7.0	7.0	9.0	4.0	0.0
100-0*1*300	300	1	12.0	1.0	7.0	7.0	9.0	4.0	0.0
100-0*2*300	300	2	12.0	1.0	7.0	7.0	9.0	4.0	0.0
80-20*0*300	300	0	9.6	1.3	9.1	7.0	9.0	4.0	2.4
80-20*1*300	300	1	9.6	1.3	9.1	7.0	9.0	4.0	2.4
80-20*2*300	300	2	9.6	1.3	9.1	7.0	9.0	4.0	2.4
100-0*0*400	400	0	12.0	1.0	7.0	7.0	9.0	4.0	0.0
100-0*1*400	400	1	12.0	1.0	7.0	7.0	9.0	4.0	0.0
100-0*2*400	400	2	12.0	1.0	7.0	7.0	9.0	4.0	0.0
80-20*0*400	400	0	9.6	1.3	9.1	7.0	9.0	4.0	2.4
80-20*1*400	400	1	9.6	1.3	9.1	7.0	9.0	4.0	2.4
80-20*2*400	400	2	9.6	1.3	9.1	7.0	9.0	4.0	2.4

PPFD=photosynthetic photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$).

Management and irrigation of hydroponic lettuce

The plants were individually placed in pots containing commercial vermiculite (Verlite) as substrate, where they remained throughout the growth stage until harvest. Nutrient solutions were prepared in containers and subsequently applied manually to each pot according to its respective treatment, using approximately 0.5 L of nutrient solution per irrigation, two to three times per week depending on moisture requirements. The pH of the nutrient solution was maintained between 5.5 and 6.0 by adjustment with sulfuric acid (H_2SO_4), and it was monitored with a pH/EC/TDS meter (HI-98130, Hanna), maintaining an electrical conductivity (EC) of 2.0 dS m^{-1} .

Experimental design and treatments

A completely randomized experimental design with a $2 \times 3 \times 3$ factorial arrangement was used, consisting of 18 treatments and five replicates. The factors corresponded to the nitrate/urea ratio, nickel concentrations, and light intensity (Table 1). Factor A consisted of the nitrate/urea ratio, with two levels: 100/0 and 80/20; factor B corresponded to nickel concentrations (0, 1, and 2 mg L^{-1}); and factor C comprised three light intensities: 200, 300, and $400 \mu\text{mol m}^{-2} \text{s}^{-1}$. The experimental unit consisted of one lettuce plant, resulting in a total of 90 experimental units. In the case of the nutrient solutions, modifications were made to the universal nutrient solution described by Steiner (1984). Photosynthetic photon

flux density (PPFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$) was measured with a ceptometer (AccuPAR LP-80, Decagon).

Growth variables

At 36 days after transplanting, the fresh weight of each lettuce plant was recorded using a precision balance (CP622, Sartorius, Germany), and root volume was determined by the water displacement technique (Böhm, 1979). The entire root system was immersed in a graduated cylinder containing a known volume of water, and the resulting displaced volume was expressed in cm^3 . Leaf and root dry weights (g) were also recorded using a precision balance. Leaf greenness index was estimated with a chlorophyll meter (SPAD-502 Plus, Konica Minolta, Japan), and leaf area (cm^2) was measured with a leaf area meter (LI-3100C, LI-COR[®] Biosciences, Lincoln, NE, USA).

Statistical analysis

An analysis of variance (ANOVA) was performed using the data obtained for the studied variables. The main factors evaluated in the three-factor design were the nitrate/urea ratio in the nutrient solution (100/0 and 80/20), nickel concentrations (0, 1, and 2 mg L^{-1}), and light intensity (200, 300, and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$), as well as their interaction. Mean comparisons were performed using Tukey's test ($p \leq 0.05$). Statistical analyses were conducted using Statistica software (StatSoft, 2004).

RESULTS AND DISCUSSION

Root volume and leaf fresh weight of hydroponic lettuce

The 100/0 nitrate/urea ratio promoted a 20% greater root volume compared with the 80/20 ratio, which is consistent with the behavior observed for leaf fresh weight, although the latter was slightly 7% higher under the treatment containing 20% urea, as shown in Table 2.

These results reflect physiological differences between the two nitrogen sources. Nitrate is the most efficient nitrogen form for promoting root growth due to its capacity to induce H^+ -ATPase activity, cell expansion, and lateral root formation (Wang *et al.*, 2023).

By contrast, urea requires prior hydrolysis, a process that temporarily reduces the immediate availability of nitrogen and may lead to ammonium accumulation, thereby negatively affecting the rhizosphere (Motasim *et al.*, 2024). Nevertheless, the slight improvement in fresh weight under the 80/20 ratio could be associated with a greater accumulation of reduced nitrogen compounds derived from ammonium assimilation originating from urea, which has been reported as a factor favoring leaf expansion in hydroponic systems when light conditions are adequate (Xu *et al.*, 2025; Yang *et al.*, 2025). In other words, nitrate maximizes root exploration, whereas low proportions of urea may favor foliar development, provided that excessive NH_4^+ levels are not generated.

Nickel showed a clear tendency to reduce both root volume and leaf fresh weight, confirming its phytotoxic effect when supplied above trace levels. Root volume decreased by 17% at 2 mg L^{-1} Ni compared with the treatment without nickel addition in the nutrient

solution ($0 \text{ mg L}^{-1} \text{ Ni}$), whereas leaf fresh weight decreased by 7% with the addition of $1 \text{ mg L}^{-1} \text{ Ni}$ relative to the treatment without nickel. However, this effect was not observed at $2 \text{ mg L}^{-1} \text{ Ni}$, where leaf fresh weight was similar to that of treatments without nickel in the nutrient solution.

These effects are explained by Ni-induced oxidative stress, which alters membrane integrity, inhibits enzymatic activity, and reduces cell division and elongation in roots (Amjad *et al.*, 2019; Kumar *et al.*, 2022). Root impairment limits the uptake of essential nutrients, which in turn affects foliar biomass. Nickel also interferes with auxin homeostasis, a hormone crucial for root system establishment (Lešková *et al.*, 2020).

An increase in light intensity generated significant increases in both root volume and leaf fresh weight. At $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$, root volume was the highest, showing a 42% increase relative to the light intensity of $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$. In contrast, leaf fresh weight was 28% higher under $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ compared with $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$. This behavior may be attributed to low light-induced leaf expansion, a phenomenon typically associated with cell elongation and greater specific leaf area, although not necessarily with a higher concentration of structural biomass.

This pattern is consistent with the findings of Pennisi *et al.* (2020) and He *et al.* (2020) in Italy and China, who observed that high light intensities stimulate root growth in lettuce cultivated under LED lighting in controlled environments. Regarding biomass accumulation, these results also agree with those described by Bantis *et al.* (2021) and Li *et al.* (2021), who demonstrated that intermediate light intensities optimize the balance among photosynthesis, transpiration, and fresh biomass accumulation in hydroponic *Lactuca sativa*, whereas high intensities may increase transpiration and respiratory cost, thereby reducing fresh weight despite enhanced photosynthesis.

The three-way interaction revealed the most pronounced contrasts in the study. The treatment consisting of the 100/0 ratio, without nickel addition, and a light intensity of $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$ produced the highest root volume, 65% greater than that observed under the treatment with 100% nitrate, no urea, $2 \text{ mg L}^{-1} \text{ Ni}$, and a light intensity of $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$. This finding demonstrates that optimal growth occurs under conditions in which nitrogen is supplied in nitrate form, nickel is absent, and the plant receives high light intensities.

This combination favors nitrate uptake, maximizes photosynthesis, and ensures an efficient pathway for carbon allocation to the roots, resulting in a highly developed root system, in agreement with previous studies in lettuce and other vegetable crops (Poorter *et al.*, 2019).

Leaf fresh weight also exhibited interactive responses. Higher values were recorded in combinations with moderate light intensities ($200\text{-}300 \mu\text{mol m}^{-2} \text{ s}^{-1}$), particularly in treatments with 20% urea and no nickel addition. This suggests that foliar biomass responds to a pattern of resource partitioning distinct from that of root volume. Under intermediate light intensities, plants prioritize leaf growth, whereas at higher intensities carbohydrate allocation toward the roots is intensified.

The results show that root and foliar growth in lettuce do not respond independently to the evaluated factors, but rather depend on their metabolic interaction. Under high light

conditions, the plant has sufficient energy to detoxify Ni, process urea, and sustain root growth. When light is limiting, the plant prioritizes chemical maintenance and reduces growth, especially when additional nickel stress is present. Carbon partitioning explains the divergence between root volume and fresh weight: high light promotes root development, whereas intermediate light favors leaf growth.

Table 2. Root volume and leaf fresh weight of hydroponic lettuce as affected by the nitrate/urea ratio, nickel, PPFD, and their interaction in a hydroponic production system under white LED lighting.

Factor	Root volume (cm ³)	Leaf fresh weight (g)
Nitrate/Urea		
100/0	40.04±1.97 a	105.90±3.85 b
80/20	32.32±0.98 b	113.48±2.61 a
Nickel ¹		
0	39.71±2.41 a	111.02±3.76 a
1	35.50±1.60 b	104.40±2.82 b
2	33.33±1.84 c	113.64±5.21 a
PPFD ²		
200	27.13±0.62 c	132.13±3.97 a
300	33.97±0.68 b	101.79±2.19 b
400	47.45±2.07 a	95.15±1.84 c
Nitrate/Urea × Nickel × PPFD		
100-0*0*200	29.80±0.20 efg	115.16±1.29 cde
100-0*1*200	28.80±1.69 fgh	115.37±5.53 cde
100-0*2*200	22.80±1.16 h	168.90±3.59 a
80-20*0*200	29.60±0.40 efg	150.48±2.23 b
80-20*1*200	27.80±1.02 fgh	123.27±2.58 c
80-20*2*200	24.00±0.45 gh	119.62±2.71 cd
100-0*0*300	35.80±0.58 cde	85.95±1.81 jk
100-0*1*300	37.80±0.86 cd	101.36±1.49 fgj
100-0*2*300	37.20±1.16 cd	91.18±2.33 hijk
80-20*0*300	31.60±1.03 def	109.87±1.90 def
80-20*1*300	29.20±0.73 fgh	104.07±4.05 efg
80-20*2*300	32.20±1.02 cdef	118.31±1.20 cd
100-0*0*400	65.20±2.18 a	99.53±1.40 fghi
100-0*1*400	51.00±2.45 b	79.11±1.64 k
100-0*2*400	52.00±1.22 b	96.52±1.48 ghij
80-20*0*400	46.28±1.89 b	105.14±2.77 efg
80-20*1*400	38.40±1.03 c	103.24±2.42 efg
80-20*2*400	31.80±1.11 def	87.35±1.22 ijk

¹Nickel (mg L⁻¹). ²PPFD=photosynthetic photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$). Means±standard error followed by identical letters within each column are not statistically different according to Tukey's test ($P\leq 0.05$).

Leaf dry weight, root dry weight, and total dry weight of hydroponic lettuce

The 100/0 nitrogen supply (nitrate only) produced a lower leaf dry weight (17%) than the 80/20 mixture, but it resulted in a greater root dry weight, with the 100/0 ratio showing an increase of 11.05% compared with the 80/20 ratio. This was reflected in a similar total dry weight between treatments (Table 3). This behavior is consistent with the greater physiological efficiency of nitrate in stimulating root growth, since it promotes auxin signaling, cell elongation, and lateral root proliferation (Vega-Matuz *et al.*, 2022). In contrast, the nitrate/urea mixture favored the accumulation of shoot biomass, which agrees with physiological models in which partially hydrolyzed urea increases the rapid availability of ammonium in the rhizosphere, thereby enhancing foliar growth in crops sensitive to the carbon/nitrogen balance (Yang *et al.*, 2025).

Regarding the different concentrations of nickel (Ni), no statistically significant differences were observed for leaf dry weight, root dry weight, or total dry weight, as shown in Table 3. However, statistically significant differences were detected among the different white LED light intensities, as also shown in Table 3. The intensity of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ produced the lowest averages for shoot dry biomass (20%), root dry weight (100%), and total dry weight (32%) compared with $400 \mu\text{mol m}^{-2} \text{s}^{-1}$. This finding confirms the positive relationship between light availability and biomass partitioning, which is attributed to greater CO_2 assimilation, carbohydrate accumulation, and protein synthesis under moderate to high light intensity (He *et al.*, 2020). Nevertheless, no symptoms of photostress were observed at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$, which is consistent with the high tolerance of lettuce under continuous white light spectra (Son and Kim, 2019). With respect to the interaction among factors, the best results were obtained with the 80/20 ratio, without nickel (0 mg L^{-1}), and under high light intensity ($400 \mu\text{mol m}^{-2} \text{s}^{-1}$), with increases of up to 100%, respectively, compared with the 100/0 ratio, a high Ni concentration (2 mg L^{-1}), and a low light intensity of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. This suggests that urea is more responsive under high-light conditions, probably due to its greater energetic cost during assimilation. Nickel intensified inhibition under low light intensity, in agreement with studies showing that nickel reduces antioxidant capacity and photosynthetic efficiency under light stress (Li *et al.*, 2024; Rehman *et al.*, 2025; Zhou *et al.*, 2024). These results confirm that productivity in hydroponic systems depends on the synchrony between nitrogen nutrition and light, and that both nitrogen assimilation and photobiological responses determine the final partitioning of biomass.

Number of leaves, leaf area, and leaf greenness of hydroponic lettuce

With respect to the nitrate/urea ratios, no statistically significant differences were observed for the variable number of leaves. However, the 80/20 nitrate:urea combination showed a significant effect on leaf area, exceeding the 100/0 ratio by 8%, which suggests a moderate contribution of urea to the expansion of lettuce leaf area. Li *et al.* (2021) reported in hydroponically grown *Lactuca sativa* that greater availability of readily assimilable nitrogen forms is associated with increases in leaf area.

This also agrees with Xu *et al.* (2025), who, in a study on hydroponic lettuce supplied with different nitrate/ammonium proportions, identified the 80:20 combination as optimal,

Table 3. Leaf dry weight, root dry weight, and total dry weight of hydroponic lettuce as affected by the nitrate/urea ratio, nickel, PPFD, and their interaction in a hydroponic production system under white LED lighting.

Factor	Leaf dry weight (g)	Root dry weight (g)	Total dry weight (g)
Nitrate/Urea			
100/0	8.57±0.25 b	2.07±0.12 a	10.64±0.31 b
80/20	10.03±0.30 a	1.83±0.10 b	11.86±0.37 a
Nickel ¹			
0	9.34±0.38 a	2.04±0.13 a	11.38±0.44 a
1	9.05±0.42 a	1.92±0.13 a	10.97±0.49 a
2	9.52±0.29 a	1.88±0.13 a	11.40±0.37 a
PPFD ²			
200	8.59±0.35 b	1.23±0.05 c	9.82±0.36 b
300	9.00±0.32 b	1.93±0.08 b	10.93±0.34 b
400	10.32±0.35 a	2.68±0.09 a	12.99±0.38 a
Nitrate/Urea × Nickel × PPFD			
100-0*0*200	8.41±0.49 abc	1.27±0.13 f	9.69±0.50 cd
100-0*1*200	7.42±1.53 c	1.24±0.14 f	8.66±1.55 d
100-0*2*200	8.88±0.46 abc	1.08±0.13 f	9.96±0.58 bcd
80-20*0*200	9.82±1.06 abc	1.22±0.13 f	11.04±1.15 abcd
80-20*1*200	8.59±0.59 abc	1.31±0.09 ef	9.90±0.58 bcd
80-20*2*200	8.44±0.59 abc	1.25±0.08 f	9.69±0.61 cd
100-0*0*300	8.00±0.50 bc	2.38±0.10 abcd	10.38±0.53 bcd
100-0*1*300	8.23±0.47 abc	1.83±0.11 cdef	10.05±0.40 bcd
100-0*2*300	9.06±0.69 abc	2.16±0.19 bcd	11.22±0.85 abcd
80-20*0*300	9.91±0.78 abc	1.80±0.13 cdef	11.70±0.87 abcd
80-20*1*300	8.82±1.18 abc	1.80±0.31 cdef	10.62±1.36 bcd
80-20*2*300	9.96±0.83 abc	1.65±0.17 def	11.62±0.79 abcd
100-0*0*400	7.90±0.83 bc	2.62±0.18 abc	10.52±0.89 bcd
100-0*1*400	9.53±0.43 abc	2.99±0.17 a	12.58±0.54 abcd
100-0*2*400	9.75±0.77 abc	3.04±0.14 a	12.78±0.84 abcd
80-20*0*400	12.01±0.50 a	2.96±0.17 ab	14.97±0.65 a
80-20*1*400	11.69±0.61 ab	2.36±0.21 abcd	14.04±0.76 ab
80-20*2*400	11.00±0.59 abc	2.12±0.21 cde	13.12±0.75 abc

¹Nickel (mg L⁻¹). ²PPFD=photosynthetic photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$). Means±standard error followed by identical letters within each column are not statistically different according to Tukey's test ($P \leq 0.05$).

significantly increasing biomass (138%). Both studies indicated that combining nitrogen forms may stimulate foliar growth by improving nitrogen use efficiency, provided that the proportion of nitrate is sufficient to prevent ammonium accumulation or phytotoxic effects. Regarding leaf greenness, no significant differences were observed when only nitrate or the nitrate:urea combination was supplied in the nutrient solution.

The Ni factor did not show significant differences for the variables number of leaves and leaf area, but it did significantly affect leaf greenness in hydroponic lettuce. The treatment with 2.0 mg L⁻¹ Ni recorded the highest greenness, with a 4% increase compared with the nutrient solution lacking nickel. The increase in leaf greenness suggests greater efficiency in nitrogen assimilation, which is consistent with the findings of Broadley *et al.* (2012), who

Table 4. Number of leaves, leaf area, and leaf greenness of hydroponic lettuce as affected by the nitrate/urea ratio, nickel, PPFD, and their interaction in a hydroponic production system under white LED lighting.

Factor	Number of leaves	Leaf area (cm ² plant ⁻¹)	Greenness (SPAD units)
Nitrate/Urea			
100/0	27.60±0.40 a	488.27±17.72 b	49.90±1.15 a
80/20	28.00±0.33 a	527.44±12.64 a	49.30±0.96 a
Nickel ¹			
0	27.70±0.47 a	500.01±21.01 a	49.87±1.31 b
1	27.80±0.51 a	513.80±18.00 a	46.93±0.98 c
2	27.90±0.37 a	509.76±18.68 a	52.01±1.41 a
PPFD ²			
200	26.07±0.43 c	627.90±7.20 a	44.00±0.98 b
300	27.60±0.31 b	481.08±14.13 b	52.08±1.14 a
400	29.73±0.32 a	414.59±6.69 c	52.72±1.18 a
Nitrate/Urea × Nickel × PPFD			
100-0*0*200	23.20±0.58 d	664.13±6.50 a	38.66±0.75 i
100-0*1*200	26.60±0.81 bcd	650.92±11.91 a	39.82±1.14 i
100-0*2*200	27.80±1.20 bc	633.45±12.63 a	43.80±1.16 fghi
80-20*0*200	28.00±0.71 b	643.12±3.71 a	42.36±1.31 ghi
80-20*1*200	24.20±0.37 cd	612.66±13.75 ab	49.24±0.74 def
80-20*2*200	26.60±0.60 bcd	563.10±8.80 bc	50.14±0.81 cde
100-0*0*300	27.60±0.60 bc	408.25±11.92 fg	54.30±0.57 bcd
100-0*1*300	27.20±0.37 bc	451.73±12.88 ef	54.82±0.96 bcd
100-0*2*300	26.40±0.51 bcd	424.05±6.75 efg	59.38±1.27 ab
80-20*0*300	29.20±0.58 ab	468.86±15.72 de	54.28±1.37 bcd
80-20*1*300	27.20±0.97 bc	514.42±23.63 cd	47.74±1.60 efg
80-20*2*300	28.00±0.95 b	619.16±9.51 ab	41.94±1.01 hi
100-0*0*400	28.20±0.80 ab	387.20±7.39 g	55.54±1.20 abc
100-0*1*400	31.80±0.49 a	389.48±9.20 g	46.72±1.41 efg
100-0*2*400	29.60±0.93 ab	385.26±4.51 g	56.08±1.25 ab
80-20*0*400	30.00±0.55 ab	428.52±6.49 efg	54.00±1.13 bcd
80-20*1*400	29.80±0.58 ab	463.59±18.91 def	43.26±1.07 ghi
80-20*2*400	29.00±0.45 ab	433.52±9.53 efg	60.72±0.87 a

¹Nickel (mg L⁻¹). ²PPFD=photosynthetic photon flux density (μmol m⁻² s⁻¹). Means±standard error followed by identical letters within each column are not statistically different according to Tukey's test (P≤0.05).

indicated that low doses of Ni stimulate nitrogen metabolism. In contrast, the variables number of leaves and leaf area showed no specific differences, which is consistent with the expectation that Ni exerts more pronounced effects on metabolism than on foliar morphogenesis directly. Regarding the increase in white LED light intensity, differentiated effects were observed: low intensity ($200 \mu\text{mol m}^{-2} \text{s}^{-1}$) produced higher leaf area values compared with high intensity ($400 \mu\text{mol m}^{-2} \text{s}^{-1}$), whereas leaf greenness and number of leaves increased progressively from the lowest intensity up to $400 \mu\text{mol m}^{-2} \text{s}^{-1}$.

This is consistent with studies indicating that lettuce maximizes leaf expansion under intermediate intensities, while chlorophyll accumulation requires higher intensities (Son and Kim, 2019). The positive response of leaf greenness and number of leaves at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ may be explained by the induction of chlorophyll synthesis and the increase in the density of the photosynthetic apparatus under greater light demand (Hogewoning *et al.*, 2010). Nevertheless, the reduction in leaf area under $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ agrees with the morphophysiological adaptation of thicker and more compact leaves under intense light.

The interaction among the three factors produced the most notable changes. The highest leaf area values (41%) were recorded under the combination of 100/0, 0 Ni, and $200 \mu\text{mol m}^{-2} \text{s}^{-1}$, indicating that low to moderate illumination favors leaf expansion when nitrogen is supplied exclusively as nitrate, compared with the combination of 100/0, 0 Ni, and $400 \mu\text{mol m}^{-2} \text{s}^{-1}$. In contrast, nitrate:urea combinations (80:20), together with nickel concentrations of 2 mg L^{-1} and a light intensity of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, tended to markedly increase leaf greenness, with values above 60 SPAD compared with the other treatments. This relationship confirms that the nitrate/urea plus nickel combination increases nitrogen assimilation efficiency, while intense light maximizes the accumulation of photosynthetic pigments. This is consistent with what has been documented for high-efficiency LED lighting systems in lettuce, where the interaction between nitrogen and light regulates plant architecture and photosynthetic capacity (Gupta and Pathak, 2025).

CONCLUSIONS

Nitrate/urea combinations under high light intensity tended to maximize parameters such as leaf area, total dry weight, and greenness, whereas the absence of nickel or unbalanced combinations reduced the effectiveness of high light intensity. This demonstrates that optimal plant performance requires a well-regulated interaction among the nitrogen source (nitrate and urea), the associated micronutrient involved in nitrogen metabolism (nickel), and light intensity. Considering the nickel concentration of 1 mg L^{-1} and its combination with the nitrate/urea nitrogen source (80/20) under a light intensity of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$, this treatment improved and optimized the development of hydroponic lettuce.

REFERENCES

- Aguilar, J. V., Lapaz, A. D. M., Bomfim, N. C. P., Mendes, T. F. S., Souza, L. A., Furlani Júnior, E., y Camargos, L. S. (2025). Photosynthetic Performance and Urea Metabolism After Foliar Fertilization with Nickel and Urea in Cotton Plants. *Agriculture*, 15(7), 699. doi.org/10.3390/agriculture15070699.
- Amjad, M., Raza, H., Murtaza, B., Abbas, G., Imran, M., Shahid, M., y Iqbal, M. M. (2019). Nickel toxicity induced changes in nutrient dynamics and antioxidant profiling in two maize (*Zea mays* L.) hybrids. *Plants*, 9(1), 5. doi.org/10.3390/plants9010005.

- Bantis, F., Smirnakou, S., Ouzounis, T., y Radoglou, K. (2021). Current status and recent achievements in the field of horticulture with the use of light-emitting diodes (LEDs). *Scientia Horticulturae*, 280, 109933. <https://doi.org/10.1016/j.scienta.2021.109933>.
- Böhm, W. (1979). Root parameters and their measurement. In *Methods of studying root systems* (pp. 125-138). Berlin, Heidelberg: Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-67282-8_12.
- Broadley, M., Brown, P., Cakmak, I., Rengel, Z., y Zhao, F. (2012). Function of nutrients: micronutrients. In *Marschner's mineral nutrition of higher plants* (pp. 191-248). Academic Press.
- Brown P. H. (2006). Ni. In: Borker, A. V. y D. J. Pilbeam (eds.). *Handbook of plant nutrition*. CRC. Press, Boca Raton, FL. pp: 329-350.
- Brown P. H., Welch R. M. and Carpy E. E. (1987). Nickel: A micronutrient essential for higher plants. *Plant Physiol.* 85: 801-803.
- Buoso, S., Lodovici, A., Salvatori, N., Tomasi, N., Arkoun, M., Maillard, A., y Zanin, L. (2023). Nitrogen nutrition and xylem sap composition in *Zea mays*: effect of urea, ammonium and nitrate on ionic and metabolic profiles. *Plant Science*, 336, 111825. doi.org/10.1016/j.plantsci.2023.111825.
- Dong, B., Wu, F., y Shen, J. (2023). Ammonium phytotoxicity and tolerance: An insight into ammonium nutrition to improve crop productivity. *Agronomy*, 13(6), 1487. doi.org/10.3390/agronomy13061487.
- Fathi, A. (2022). Role of nitrogen (N) in plant growth, photosynthesis pigments, and N use efficiency: A review. *Agrisost* ISSN 1025-0247, 28, 1-8. <https://doi.org/10.5281/zenodo.7438164>.
- Gupta, B., y Pathak, G. C. (2025). Role of nickel in plants: A review. *Indian J. Appl. Pure Bio*, 40, 2175-2181.
- Guzmán M., Ambar R., Cruz., Orestes, Valdés C.R., y Valdés, H, Pedro A. (2021). Evaluación de la contaminación por metales pesados y su acumulación en plantas de lechuga (*Lactuca sativa* L.). *Cultivos Tropicales*, 42(4), e03. [de.http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S02585936202100040003&lng=es&tlng=es](http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S02585936202100040003&lng=es&tlng=es).
- He, D., Kozai, T., Niu, G., Zhang, X., y Guan, Y. (2020). LED lighting and growth responses of leafy vegetables. *Scientia Horticulturae*, 263, 108527. <https://doi.org/10.1016/j.scienta.2019.108527>.
- He, D., Zhang, S., Lu, S., y Liu, H. (2020). LED lighting and growth responses of leafy vegetables. *Scientia Horticulturae*, 263, 108527. <https://doi.org/10.1016/j.scienta.2019.108527>.
- Hogewoning, S. W., Trouwborst, G., Maljaars, H., Poorter, H., van Ieperen, W., y Harbinson, J. (2010). Blue light dose—responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. *Journal of experimental botany*, 61(11), 3107-3117. <https://doi.org/10.1093/jxb/erq132>.
- Hong, Y., Liu, R., Xiang, W., Lei, P., y Fang, X. (2025). Emergent Plants Improve Nitrogen Uptake Rates by Regulating the Activity of Nitrogen Assimilation Enzymes. *Plants*, 14(10), 1484. doi.org/10.3390/plants14101484.
- Jurga, A., Janiak, K., Wizimirska, A., Chochura, P., Muszyński-Huhajło, M., Ratkiewicz, K., y Podstawczyk, D. (2021). Resource recovery from synthetic nitrified urine in the hydroponic cultivation of lettuce (*Lactuca sativa* var. *capitata* L.). *Agronomy*, 11(11), 2242. <https://doi.org/10.3390/agronomy11112242>.
- Kabata, P., A. (2000). Trace Elements in Soils and Plants. Third edition. CRC Press. 413p.
- Karwowska, M., y Kononiuk, A. (2020). Nitrates/nitrites in food—Risk for nitrosative stress and benefits. *Antioxidants*, 9(3), 241. doi.org/10.3390/antiox9030241.
- Khan, N. K., Watanabe, M., y Watanabe, Y. (2000). Effect of partial urea application on nutrient absorption by hydroponically grown spinach (*Spinacia oleracea* L.). *Soil Science and Plant Nutrition*, 46(1), 199-208. <https://doi.org/10.1080/00380768.2000.10408775>.
- Khoshgoftarmanesh, AH, Hosseini, F. y Afyuni, M. (2011). Efecto de la suplementación con níquel sobre el crecimiento, la actividad de la ureasa y las concentraciones de urea y nitrato en lechuga cultivada con diferentes fuentes de nitrógeno. *Scientia Horticulturae*, 130(2), 381-385.
- Kumar, S., Wang, M., Liu, Y., Fahad, S., Qayyum, A., Jadoon, S. A., y Zhu, G. (2022). Nickel toxicity alters growth patterns and induces oxidative stress response in sweetpotato. *Frontiers in Plant Science*, 13, 1054924. doi.org/10.3389/fpls.2022.1054924.
- Lešková, A., Zvarík, M., Araya, T., y Giehl, R. F. (2020). Nickel toxicity targets cell wall-related processes and PIN2-mediated auxin transport to inhibit root elongation and gravitropic responses in Arabidopsis. *Plant and Cell Physiology*, 61(3), 519-535. doi.org/10.1093/pcp/pcz217.
- Li, J., Wu, T., Huang, K., Liu, Y., Liu, M., y Wang, J. (2021). Effect of LED spectrum on the quality and nitrogen metabolism of lettuce under recycled hydroponics. *Frontiers in Plant Science*, 12, 678197. <https://doi.org/10.3389/fpls.2021.678197>.
- Li, J., Zhang, Z., y Wang, Q. (2024). Physiological and molecular bases of the nickel toxicity responses in tomato. *Stress Biology*. <https://doi.org/10.1007/s44154-024-00162-0>.

- Loconsole, D., Cocetta, G., Santoro, P., y Ferrante, A. (2019). Optimization of LED Lighting and Quality Evaluation of Romaine Lettuce Grown in An Innovative Indoor Cultivation System. *Sustainability*, *11*(3), 841. <https://doi.org/10.3390/su11030841>.
- Motasim, A. M., Samsuri, A. W., Nabayi, A., Akter, A., Haque, M. A., Sukor, A. S. A., y Adibah, A. M. (2024). Urea application in soil: processes, losses, and alternatives—a review. *Discover Agriculture*, *2*, 42. doi.org/10.1007/s44279-024-00060-z.
- Nieves-Cordones M, Rubio F and Santa-María GE (2020) Nutrient Use-Efficiency in Plants: An Integrative Approach. *Front. Plant Sci*. 11:623976. doi: 10.3389/fpls.2020.623976.
- Pennisi, G., Blasioli, S., Cellini, A., Maia, L., Crepaldi, A., Braschi, I., Spinelli, F., Nicola, S., Fernandez, J. A., Stanghellini, C., y Orsini, F. (2020). Unraveling the role of red:blue LED lights on resource use efficiency and nutritional properties of indoor grown lettuce. *Frontiers in Plant Science*, *11*, 592. <https://doi.org/10.3389/fpls.2020.00592>.
- Poorter, H., Fiorani, F., Stitt, M., et al. (2019). The plant phenotype: From closer to global-scale views. *Plant Journal*, *97*(1), 19–40. <https://doi.org/10.1111/tpj.14171>.
- Rehman, M., Ali, B., Salam, A., Zaynab, M., Haider, Z., Yasin, M. U., y Gan, Y. (2025). Nickel Stress Modulates Growth Dynamics, Disrupts Redox Homeostasis, and Induces Ultrastructural Damage in Maize. *Crop Design*, 100122. doi.org/10.1016/j.crope.2025.100122.
- Reyes-Rosas, A. , Moreno-Garza, A. P. , Gomez-Ramirez, E. , Mounzer, O. , Khamkure, S. , y Lara-Viveros, F. M. . (2025). Efecto de luz LED complementaria intermitente sobre el crecimiento de lechuga bajo invernadero. *Revista Mexicana De Ciencias Agrícolas*, *16*(30), e4044. <https://doi.org/10.29312/remexca.v16i30.4044>.
- Rizwan, M., Usman, K., Alsafran, M., Jabri, HA, Samreen, T., Saleem, MH y Tu, S. (2022). La toxicidad del níquel interfiere con la absorción de $\text{NO}_3^- / \text{NH}_4^+$ y la actividad de las enzimas del metabolismo del nitrógeno en el arroz (*Oryza sativa* L.). *Plants* , *11*(11), 1401.
- Son, K. H., y Kim, D. (2019). Effects of LED light spectra and intensities on growth, photosynthesis and phytochemicals in lettuce. *Acta Horticulturae*, 1256, 269-276. <https://doi.org/10.17660/ActaHortic.2019.1256.35>
- Son, K., y Kim, D. (2019). Light management in lettuce under LED. *Acta Horticulturae*, 1246, 39-46. <https://doi.org/10.17660/ActaHortic.2019.1246.5>
- StatSoft, inc. 2004. STATISTICA (data analysis software system), version 7. www.statsoft.com.
- Steiner, A. A. 1984. The universal nutrient solution. Sixth Int. Congr on Soilless Culture. ISOSC Proceeding. *The Netherlands*. 633-649 pp.
- Vega-Matuz, R. et al. (2022). Nitrate nutrition and root development. *Journal of Plant Nutrition*. <https://doi.org/10.1080/01904167.2021.2022945>
- Villegas, T O. G., Dominguez, P. M. L., Martínez, J. P y Aguilar, C. M. (2015). Cobre y Níquel, microelementos esenciales en la nutrición vegetal. *ECORFAN*®, *2*(2): 285-295.
- Wang, Y., Xing, J., Wan, J., Yao, Q., Zhang, Y., Mi, G., y Zhang, M. (2023). Auxin efflux carrier ZmPIN1a modulates auxin reallocation involved in nitrate-mediated root formation. *BMC Plant Biology*, *23*(1), 74. doi.org/10.1186/s12870-023-04087-0.
- Xu, J., Che, G., Yan, J., Luo, Y., Good, A., Yuan, T., ... y Shizumi, K. (2025). Optimizing nitrogen form ratio for enhanced nutrient uptake and lettuce performance in hydroponic systems. *Bioresource Technology Reports*, 102384. doi.org/10.1016/j.biteb.2025.102384
- Yang, Z., Yan, H., Liu, H., Yang, L., y Mi, G. (2025). Enhancing crop nitrogen efficiency: The role of mixed nitrate and ammonium supply in plant growth and development. *Biology*, *14*(5), 546. <https://doi.org/10.3390/biology14050546>.
- Zhou, X., An, Y., Qu, T., Jin, T., Zhao, L., Guo, H., y Wang, W. (2024). Effects of Ni and Cu stresses on morphological and physiological characteristics of Euphorbia marginata seedlings. *Agronomy*, *14*(6), 1223. <https://doi.org/10.3390/agronomy14061223>.