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Effect of nickel addition on yield in hydroponic lettuce (*Lactuca sativa*)

Efecto de la adición de níquel en la producción de lechuga hidropónica (*Lactuca sativa*)

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Abstract

Lettuce is a horticultural plant that has been welcome in the diet of consumers worldwide. Unlike others, it is a vegetable that has a low flavor and contains few percentages of nutrients. However, it is 96 % water and provides some fiber and folic acid to meals. The experiment consisted of the foliar application of nickel sulfate in different concentrations every 15 days from transplanting to the physiological crop maturity. Yield values were determined in the plants: fresh root weight, leaf diameter, stem diameter, and root length compared with the means in the fresh root weight. The highest average was obtained with the 0.0018 g nickel concentration, with which a 51.666 g average was obtained. The 0.0016 g concentration showed a 146.66 g mean in the fresh plant weight variable as well as better results in stem diameter with a 1.5 mm diameter. The same treatment presented superior responses for plant leaf diameter with 29 cm. The long root variable obtained an average of 39.6 cm with a 0.0018 g concentration. The 0.0016 g foliar concentration resulted in better yields in fresh plant weight, stem diameter, and leaf diameter.

Hydroponics, Agriculture, Sustainable, Water and nickel

Resumen

La lechuga es una planta hortícola que ha sido bien aceptada en la dieta de los consumidores de todo el mundo. Es una hortaliza que, a diferencia de otras, escasea en sabor y contiene pocos porcentajes de nutrientes. Sin embargo, se compone en un 96 % de agua y aporta algo de fibra y ácido fólico a las comidas. El experimento consistió en la aplicación foliácea de sulfato de níquel, en diferentes concentraciones, cada 15 días desde el trasplante hasta la madurez fisiológica del cultivo. En las plantas se determinaron los valores de rendimiento: peso fresco de la raíz, diámetro de la hoja, diámetro del tallo y longitud de la raíz. En comparación con las medias en el peso fresco de la raíz, el promedio más alto se obtuvo con la concentración de 0.0018 g de níquel, con lo cual se promediaron 51.666 g. La concentración de 0.0016 g observó una media de 146.66 g en la variable peso fresco de la planta, al igual que mejores resultados en el diámetro del tallo, con un promedio de 1.5 mm. El mismo tratamiento presentó mejores respuestas al diámetro de la hoja de la planta, con 29 cm. La variable raíz larga obtuvo un promedio de 39.6 cm con la concentración de 0.0018 g; mientras que la concentración de 0.0016 g por vía foliar arrojó mejores rendimientos en el peso fresco de la planta, diámetro de tallo y diámetro de hoja

Hidroponía, Agricultura, Sostenible, Agua y níquel

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Introduction

Insufficient intake of dietary micronutrients may reflect low mineral contents in vegetables. So far, 22 trace elements, essential for proper human growth and development, have been determined. Some of these elements are essentially required in small amounts, and their deficiency is considered unlikely or even unknown by the final consumer (Stein, 2009).

According to Kim *et al.* (2016), internationally, lettuce is rich in fiber, fatty acids, amino acids, vitamins A, C, E, B1, B_2 and B_3, proteins and minerals (Cu, Al, Na, Mg, K and Ca), whose leaves are especially edible (Rijk, 2014). It is an outstanding crop for small and medium producers in the high plateau who have areas with low availability of nutrients, humidity, and organic matter (Rodríguez, 2014). Hydroponics has been widely used for experimentation in plant mineral nutrition and further classified as the most intensive and high-tech current horticultural production method in reduced spaces. Generally, this production characteristic is attributed when including a significant capital investment, successfully applied in developed countries (Institute for Technological Innovation in Agriculture, INTAGRI, 2017a).

One of the most widely used strategies that seeks phyto-improvement in plants is known as biofortification. It is carried out to increase the number of micronutrients in grains, roots, and tubers, allowing the nutritional conditions of the producer's family nucleus to improve thanks to self-consumption. Today in the recirculating water system (hydroponics), demands for nutrient formulations called nutrient solutions are made (Institute for Technological Innovation in Agriculture, INTAGRI, 2017b). They resemble the nutrients present in the soil and, by using them in hydroponics, the plant receives them in an optimal and balanced way thus, ensuring a good yield and commercial quality.

Given the relevance of agricultural products in the intake of these nutrients, in recent years, it has been investigated and put into practice a novel way of enriching plant products intended for human consumption called biofortification. It is defined as the process of increasing the concentration of essential elements in the edible aerial part of harvested products through agronomic intervention (Ríos-Ruiz, 2013; López-Diago, 2022).

Although some conventional breeding methods exist, they are considered laggards because they limit maneuverability when facing the high requirements of today's consumers. Thus, genetic engineering plays a leading role in new methodologies for developing well-fortified foods that improve the nutritional conditions of the most vulnerable populations (Rodríguez-Penagos, 2015).

Currently, production does not represent a serious problem; however, society demands high quality in these products and needs them to have properties both to reduce hunger and satisfy human nutritional needs. In general, all human beings require more than 22 mineral elements for correct development in the system (Ríos-Ruiz, 2013).

The information generated will contribute to mitigating nutritional imbalances, proposing diet diversification, mineral supply, fortification of processed foods (food fortification), and, as we have seen, crop biofortification programs. Such is its acceptance that numerous countries have identified biofortification as a key tool to improve the population's nutritional status and have integrated it into their agricultural policies (Funes *et al.*, 2015). Therefore, the mission, vision, and objectives are presented, which suggest increasing the levels of scientific and technological development for such programs, as well as characterizing agri-food products (food for human consumption).

Kutman *et al.* (2013b), mention that nickel was previously considered a secondary micronutrient. But before 2003, the United States Department of Agriculture declared it an essential nutrient, as different studies demonstrated its importance in plant nutrition. In the beginning, there was speculation about its indispensability in some leguminous plants. Then in 1987, a study conducted by Patrick Brown of the University of Davis characterized it as an essential nutrient for non-leguminous plants.

This element is absorbed by plants in the form of the divalent cation Ni2+. Required by plants with low concentrations, it is also used for nitrogen conduction metabolism and plant germination; this strengthens physiological processes. Cakmak (2014), argues that nickel deficiency slightly inhibits the action of urease; this explains that the accumulation of urea causes necrotic spots on leaves. The author also refers that the metabolism of ureides, amino acids, and organic acids is affected, accumulating oxalic and lactic acid in leaves.

He also states that urease is an enzyme of great importance in plant nutrition, where urea is an essential nitrogen source. This enzyme is responsible for hydrolyzing urea into ammonia so that it can be assimilated by the plant (Kutman *et al*., 2013a).

In the present study, the yield, root fresh weight, leaf diameter, stem diameter, and root length of lettuce crop in a recirculating water system (hydroponics) with Parris Island variety were evaluated by foliar applications of nickel sulfate dissolved in one L of water.

Materials and methods

The experiment was conducted at the Technological University of Rodeo, in the municipality of Rodeo, Durango, inside the semi-automated greenhouse, in the NFT Hydroponic Systems Area, which is suitable for producing leafy vegetables with medium technology. This is located at Carretera Federal Pan-American km 159.4, México 45 SN, 35760 Rodeo, Durango.

Preparation of nickel (Ni) to be applied to the hydroponic lettuce plant

Application doses were prepared in the form of plant assimilation and mg, weighed on a digital scale; subsequently in one L of water and with one mL of dispersant in three sprinklers marked with the treatment number the mineral was diluted; three applications were made every 15 days after the seedling was established in the NFT system. It is worth mentioning that the doses of foliar application of the element were determined based on the assimilation form of the plant (nickel).

Preparation of the research area

The area where the work took place was conditioned, giving the NFT hydroponic system and its surroundings maintenance.

Sowing

Sowing was conducted, placing one seed per hole in three polyethylene trays without cavities easily watered with the help of a watering can. The containers remained covered with three plastic bags to avoid excessive light. Once the emergency occurred, the seedbed was uncovered to prevent etiolation of the plant.

Disinfection of the NFT hydroponic system

For the disinfection of the NFT hydroponic system, 10 % chlorine was used to eliminate residues from the previous harvest and any disease or parasites present.

Plant nutrition

In this activity, the nutrient solution in the development process in the seedbed area was established (Table 1).

Formulas	Nutritive solution for 100 L of water	Concentration	Nutritive solution for the hydroponic system
Calcium nitrate	Ca (NO_3) 2, Ca=40, N=14 and O=16(3)	143 g, 164 g/mol	0.8715 mol
Magnesium sulfate	$MgSO_4$, Mg=24, S=32 and O=16 (4)	57g, 120 g mol	0.4736 mol
Monopotassium phosphate	KH_2PO_4, K=39, N=30 and O=16 H=1	28.5 g, 136 g mol	0.2058 mol
Monoammonium sulfate	$(NH_4)2SO_4$, N=14*2, H=1*4*2, S=32*4 and O=16*4	30 g, 228 g mol	0.1314 mol

Table 1 Steiner nutrient solution in hydroponic lettuce plants

Planting

It was carried out 32 days after germination, when the plant reaches between 10 and 12 cm in height to the PVC tubes (NFT System) so that they exceed the holes of the tube.

Crop nutrition

A Steiner Nutrient Solution (SN) proposed by Steiner in 1984 was made; and applied to the system for one week based on the water analysis for the crop with an initial concentration of 50 %, in a 450 L container of this solution. Afterward, this was increased to 75 %, and applied for two weeks; finally, the solution was subjected to 100 % in a container with 19 L of water.

At the end of one week of application, the SN was decreased to 25 %, before harvesting, to prevent the lettuces from becoming bitter. The fertilizers used were MKP (monopotassium phosphate), $CaNO_3$ (calcium nitrate), $MgNO_3$ (magnesium nitrate), KNO_3 (potassium nitrate), KSO_4 (potassium sulfate), NH_4SO_4 (ammonium sulfate), micronutrients, and the addition of nickel (Ni) (Quipuscoa-Juarez, 2022).

Statistical analysis

Data were subjected to analysis of variance (Anava), and significant differences were identified by the comparison test among means, with a significance level of 95 % and a value of 0.05. Statistical analyses were undertaken using SPSS software, version 15 (Rivadeneira *et al.*, 2020).

Results and discussion

Analysis of variance and comparison of means with single sample test on root fresh weight

Comparing means in root fresh weight, the highest one was observed with 0.0018 g of nickel, whose mean was 51.666 g. The absolute control treatment and the treatment with 0.0016 g were similar in the contribution of nickel sulfate (Table 2).

Concentration	Treatments	Degrees of freedom	Significance (bilateral)	Difference of means	95 % confidence interval for difference
0 g	43.95	2	0.000	43.95	39.64 48.25
0.0014 g	6.2597	2	0.024	37.61	11.76 63.47
0.0016 g	13.862	2	0.005	40.28	27.78 52.78
0.0018 g	3.0676	2	0.091	51.61	20.77 124.01

Table 2 Analysis of variance in concentrations for root fresh weight with significant difference of 95 %

When comparing these results with those obtained in Anava, the contribution was 0.0018 g of nickel (Table 3). It was observed that the higher the nickel concentration, the higher the fresh weight will be obtained, but in minimum concentrations to avoid toxicity to the plant, as expressed by Wood *et al.* (2004a, 2004b, 2004c), this is because the levels considered required are small such as 0.001 $mg.kg^{-1}$ dry weight.

Concentration	Number	Mean square	Standard deviation	Standard error of the mean
0 g	3	44	1.73	1
0.0014 g	3	37.6	10.40	6.00
0.0016 g	3	40.3	5.03	2.90
0.0018 g	3	51.6	29.14	16.82

Table 3 Comparison of means for concentrations with plant fresh weight variable

However, Kutman *et al*. (2013b), conducted experiments on barley, potato, and broad bean crops, demonstrating yield increases with the foliar application of nickel. Villegas-Torres *et al*. (2015), stated some plants accumulate metals and are a viable alternative, but the purpose here is to increase this yield even more with the application of microorganisms with two functions: plant growth promoters and high availability for metals in their metabolism. At low concentrations, nickel can replace these applications of microorganisms in plants, though (Birgi *et al*., 2022).

Analysis of variance and comparison of means with single sample of fresh weight test

The highest averages in the mean comparison observed a stable behavior, employing the concentration of 0.0016 g of nickel, where the mean was 146.66 g in the absolute control treatment and the 0.0018 g treatment, which are statistically similar in the contribution of nickel sulfate in fresh weight as established in Table 4.

Concentration	Treatments	Degrees of freedom	Significance (bilateral)	Difference of means	95 % confidence interval for difference
0 g	43.95	2	0.000	43.95	39.64 48.25
0.0014 g	6.259	2	0.024	37.61	11.7663.47
0.0016 g	13.86	2	0.005	40.28	27.78 52.78
0.0018 g	3.067	2	0.091	51.61	20.77 124.0

Table 4 Analysis of variance in concentrations for plant fresh weight with a significant difference of 95 %

In the present analysis, the contribution in the absence of nickel yielded a weight of 15.11 g, the same with 0.0016 g and 12.62 g among the treatments (Table 5).

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Concentration	Number	Mean square	Standard deviation	Standard error of the mean
0 g	3	128.666	14.742	8.511
0.0014 g	3	137.333	29.501	17.032
0.0016 g	3	146.666	20.108	11.609
0.0018 g	3	124.333	20.816	12.018

Table 5 Comparison of means in concentrations with leaf diameter variable

On the other hand, when a low micronutrient concentration is present in seeds, the yield in weight decreases by 50 %; in addition, the foliar growth of the plants is affected (Kutman *et al*., 2013b). It shows that treatments with low seed concentration of nickel reflect inefficiency and that foliar applications showed favorable results in the plant. As referenced by Olivares-Arias *et al.* (2014), the nickel entered by foliar way are beneficial in the human system.

Analysis of variance and comparison of means with single sample test on leaf diameter

When comparing leaf diameter, the highest mean was obtained with the concentration of 0.0016 g, with a value of 29 g, and for treatments 0 in the absence of nickel and 0.0018 g, where the mean was 27.5 and 27.6 g; similar in the contribution of nickel in leaf diameter (Table 6).

Concentration	Treatments	Degrees of freedom	Significance (bilateral)	Difference of means	95 % confidence interval for the difference
0 g	15.111	2	0.026	27.61	7.691 47.541
0.0014 g	8.060	2	0.008	22.95	13.993 31.906
0.0016 g	12.629	2	0.002	28.95	22.377 35.522
0.0018 g	10.340	2	0.015	27.45	22.377 35.522

Table 6 Analysis of variance in concentrations for leaf diameter with a significant difference of 95 %

It is to argue that the principal factors affecting the plant are leaf absorption, which safeguards leaf characteristics (cuticle composition, stomata, and trichomes, etc.); the phenological stage; the mobility of nutrients within the plant, or the presence of stress (Ponciano-Ramos, 2022). For this reason, Li *et al*. (2012), reported high levels of 2.34±0.27 µg/g. in the rice crop. It occurs because the plant is in direct contact with water that may come from contaminated effluents.

The cuticle is mainly made of a polar compound, such as waxes, cutin, and molecules, which makes it an essentially hydrophobic membrane where the permeability rate of the leaf is completed. But there is no research arguing the loss in leaf diameter of plants in hydroponic conditions INTAGRI (2014), with high nickel mobility in the xylem and phloem. Because of the above, nickel is related to tissues, where it is transported in the aerial parts of plants (Yusuf *et al.*, 2011).

Comparison of means with stem diameter

In the comparison of means of stem diameter, it was obtained that the highest average in the treatments with 0 in the absence of nickel and the treatment 0.0016 g, with a value of 1.5 mm are statistically equal; the lowest was obtained by the concentration of 0.0018 g with a value of 1.23 mm in the contribution of nickel in stem diameter (Table 7). An investigation by Rodríguez-Jiménez *et al*. (2016), showed that nickel is principally distributed in epidermis cells in stems and leaves of the *hyperaccumulators Alyssum bertolonii*, *Alyssum lesbiacum*, and *Thlaspi goesingense*. It is also distributed in vacuoles rather than in the cell wall. However, 67 % to 73 % of the nickel is sheltered in the leaves, as demonstrated in the cell walls of *Thlaspi goesingense*.

Concentration	Number	Mean square	Standard deviation	Standard error of the mean
0 g	3	1.5	0.17320508	0.1
0.0014 g	3	1.36	0.11547005	0.06
0.0016 g	3	1.5	0	0
0.0018 g	3	1.23	0.11547005	0.14

Table 7 Comparison of means for concentrations with stem diameter variable

The Anava analysis established a value of 0.05 at the concentration of 0.0014 g of nickel and obtained a value of 1.5 mm as the absolute control (0 g) in the absence of nickel among the treatments. The concentrations of 0.0018 g and 0.0016 g are statistically equal as shown in Table 8.

Concentration	Treatments	Concentration	Treatments	Degrees of freedom	Significance (bilateral)
0 g	14.5	2	0.004	14.5	1.01 1.880
0.0014 g	19.75	2	0.002	1.37	1.029 1.603
0.0016 g	8.14425855	2	0.014	1.18	0.558 1.808
0.0018 g	14.5	2	0.015	14.5	1.0191.880

Table 8 Analysis of variance in concentrations for stem diameter with a significant difference of 95 %

Bryson and Gretchen (2014), expose that usually toxicities occur in woody plants. In turn, the present study experimented with lettuce plants to contemplate the behavior of nickel additions. Similarities were also observed in a work that reported tissue levels exceeding 80 and 120 ppm. Sensitive plants, e.g., tomato, may contain some slight toxicities above 10 ppm in the tissue part.

Rodriguez-Jimenez *et al*. (2016), argue that the contribution of transported nickel in stems and leaves is mainly localized in vacuoles, cell walls, and epidermal trichomes associated with citrate, malate, and malonate.

Comparison of means with root length

In the comparison of means, the highest increase in root length obtained by concentration was observed with 0.0018 g of nickel, with a value of 39.6 cm and, subsequently, the absolute control (0 g) in the absence of nickel, with a value of 35 cm is the lowest in the contribution of nickel in root length (Table 9).

Concentration	Number	Mean square	Standard deviation	Standard error of the mean
0 g	3	35	8.660	5
0.0014 g	3	29	4.041	2.333
0.0016 g	3	30	4.358	2.516
0.0018 g	3	39	6.350	3.666

Table 9 Comparison of means between concentrations with root length variable

In Berkheya coddii, Ni^{2+} uptake was inhibited by Ca^{2+} and Mg^{2+}. However, Ca^{2+} and Mg^{2+} do not compete in the uptake of Ni^{2+} present in barley (*Hordeum vulgare* L.) roots; in matters of Zn^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+}, and Pb^{2+} ions inhibit Ni^{2+}. Among these Zn^{2+} and Cu^{2+} were evidenced to be very competitive, Co^{2+} was slightly shown, and Cd^{2+} and Pb^{2+} did not indicate to be competitive among the others (Walsh & Orme-Johnson, 1987; Mulrooney & Hausinger, 2003; Augusto, 2022).

For analysis of variance (Anava), the concentration of 0.0018 g nickel observed an increase in root length, with 39.61 cm, followed by the concentration of 0.0014 g and 0.0016 g nickel, with a 29.9 cm root increase. In treatments, they are statistically equal, as represented in Table 10.

Concentration	Treatments	Degrees of freedom	Significance (bilateral)	Difference of means	95 % confidence interval for the difference
0 g	6.99	2	0.019	34.95	13.436 56.463
0.0014 g	12.55	2	0.006	29.28	19.243 39.322
0.0016 g	11.9009232	2	0.006	29.95	19.121 40.778
0.0018 g	10.8045455	2	0.008	39.61	23.840 5.393

Table 10 Analysis of variance in concentrations for root length with a significant difference of 95 %

As pointed out by León and Sepulveda (2012), ion uptake at the root epidermis and movement from the epidermis to the endodermis is a function of apoplexy and translocation of nickel and copper present in the root to the leaves through the transporter duct, the xylem. These activities are carried out slowly by the current of transpiration, evidenced by the present investigation in the concentration with 39.61 cm, whose increase was the highest towards the roots of the lettuce plant. The root showed a higher increase in the present variables.

Conclusions

In the comparison of means in root fresh weight, the highest average was obtained with the concentration of 0.0018 g nickel, with the highest value of 51.666 g. The best performance was observed there.

In the case of comparison of means with the concentration of 0.0016 g of nickel, a mean of 146.66 g in plant fresh weight was obtained. Likewise, the same treatment showed better results in stem diameter, with a mean of 1.5 mm. Finally, the same treatment established better responses in plant leaf diameter with 29 cm.

In root length, a mean of 39.6 cm was observed with a concentration of 0.0018 g of nickel addition.

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