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Do vitamins affect the morphophysiology of lettuce in a hydroponic system?[1]

Vitaminas afetam a morfofisiologia da alface em sistema hidropônico?

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HIGHLIGHTS:

The combined use of thiamine and niacin has a synergistic effect on the morphophysiology of lettuce grown in a hydroponic system.
The application of combined vitamins results in gains of more than 30% over the fresh mass of lettuce under a hydroponic system.
The isolated application of thiamine can reduce the growth characteristics of hydroponic lettuce.

ABSTRACT: Technologies once applied exclusively in conventional systems have maximized gains in hydroponic systems; however, they still need information for proper use. Thus, the study aimed to evaluate the effect of foliar application of thiamine and niacin on growth and gas exchange activities in two lettuce cultivars in an ebb and flow hydroponic system. The completely randomized design (CRD) was used with four treatments and eight replications. The treatments were as follows: T1 - control, T2 - thiamine at a concentration of 200 mg L^{-1}, T3 - niacin at 200 mg L^{-1}, and T4 - thiamine and niacin combined at 100 mg L^{-1} of each vitamin. Each experimental plot consisted of a lettuce plant. The combined application of thiamine and niacin stood out, increasing the activity of gas exchange of lettuce plants and increasing by about 30% the fresh mass, concerning the control, for both cultivars, followed by the isolated application of niacin. For thiamine, the application alone did not result in significant changes. The vitamins affect the morphophysiology of lettuce, where the use of niacin has the potential for use in hydroponic production systems. Its combined use with thiamine increases this potential, with a synergistic effect between the compounds being verified.

Key words: *Lactuca sativa* L., biostimulant, gas exchange, soilless cultivation

RESUMO: Tecnologias outrora aplicadas exclusivamente em sistemas convencionais, têm maximizado os ganhos em sistemas hidropônicos, porém, necessitando ainda de informações para uma adequada utilização. Assim, o estudo teve como objetivo avaliar o efeito da aplicação foliar de tiamina e niacina sobre o crescimento e atividades de troca gasosa em duas cultivares de alface em sistema hidropônico de fluxo e refluxo. O delineamento experimental utilizado foi o inteiramente casualizado (DIC), com quatro tratamentos e oito repetições. Os tratamentos foram: T1 - controle; T2 - tiamina em concentração de 200 mg L^{-1}; T3 - niacina em concentração de 200 mg L^{-1}; T4 - tiamina e niacina combinadas, em concentração de 100 mg L^{-1} de cada vitamina. Cada parcela experimental foi constituída por um vaso, contendo uma muda de alface. A aplicação conjunta de tiamina e niacina elevou as trocas gasosas das plantas de alface, aumentando em cerca de 30% a massa fresca, em relação ao controle, para ambas as cultivares, seguida pela aplicação isolada de niacina. Para a tiamina, a aplicação de forma isolada não resultou em alterações significativas. As vitaminas afetam a morfofisiologia da alface, onde o uso da niacina tem potencial para uso em sistemas de produção hidropônicos, sendo esse potencial aumentado pelo seu uso conjunto com a tiamina, verificando-se um efeito sinérgico entre os compostos.

Palavras-chave: *Lactuca sativa* L., bioestimulante, trocas gasosas, cultivo sem solo

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Introduction

As an expanding technique, hydroponics is introduced in environments with different levels of technology, which results in variations in the characteristics present in the system, which can affect growth and production (Sambo et al., 2019; Batista et al., 2021; Costa et al., 2021). In this sense, obtaining practices that aim to improve the use of the resources involved in these systems can result in significant gains for the producer.

Technologies proven to be effective in conventional cultivation are now being explored in hydroponics, such as inoculation with bacteria that promote plant growth, which can result in mass gains in lettuce (Moreira et al., 2022) and basil (Kolega et al., 2020), application of beneficial elements such as Si, which also resulted in an increase in fresh mass in basil plants (Li et al., 2020), and different types of biostimulants, capable of promoting plant growth and quality (Parađiković et al., 2019). However, there is a range of compounds that need studies aiming at their application in hydroponic systems, including vitamins.

Among the vitamins currently studied for application in production systems, those belonging to the B complex, such as niacin and thiamine, stand out. Its foliar application results in a series of morphophysiological changes, such as increased photosynthetic capacity under stressful conditions (Ramos et al., 2021; Vendruscolo et al., 2022), better development of vegetative and reproductive organs (Colla et al., 2021) and increased levels of photosynthetic pigments (Vendruscolo et al., 2021). Some studies also show an increase in the accumulation of energy and nutrient reserves (Kaya et al., 2015).

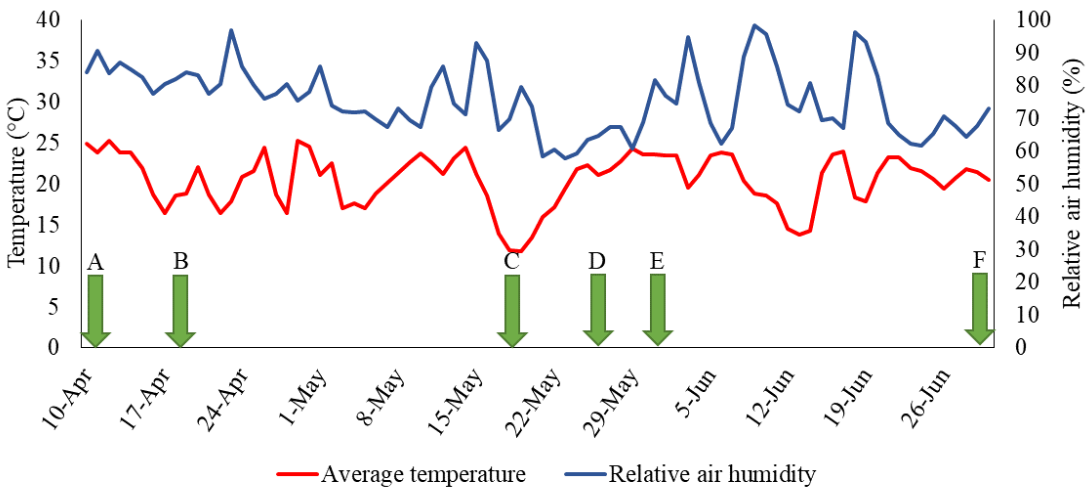
Under the hypothesis that vitamins can help to obtain gains on morphophysiological characteristics in hydroponic crops, the study aimed to evaluate the effect of foliar application of thiamine and niacin on growth and gas exchange activities in two lettuce cultivars in an ebb and flow hydroponic system.

Material and Methods

Two experiments were carried out in an acclimatized greenhouse, with a dimension of 14.64 × 6.40 × 3.5 m, 3.66 × 3.20 m, closed on the roof and side by a low-density polyethylene film (LDPE) 150 microns, light, double layer, with 35% shading aluminized thermos-reflective screen (Aluminet®) under the cover film and pad/fan Humil Cool (CELDEX®) climate control system, the environment has six internal metallic benches with dimensions of 1.10 m × 5.0 m and 0.80 m high, and concrete floor. The structure belongs to the Experimental Farm of the Mato Grosso do Sul State University (Universidade Estadual de Mato Grosso do Sul - UEMS), in Cassilândia, MS, at 19º05'46"S and 51º48'50"W, and altitude of 521 m. For the first experiment, the cultivar Pira Roxa was used, cultivated between April and May 2022, while for the second experiment, the cultivar Milena was used between May and June (Figure 1).

The ebb and flow hydroponic system was used, consisting of 2.0 m long, 0.15 m wide, and 0.05 m high gutters, flooded with a nutrient solution (18% N, 8% P, 30% K, 3% S, 3% Mg, 0.14% Fe, 0.04% B, 0.04% Mn, 0.03% Cu, 0.019% Mo, 0.006% Ni, 0.002% Co, and 15% Ca) for leafy vegetables (Plantpar®) three times a day for periods of 11 min. Black polyethylene pots of 0.5 mL, filled with commercial peat substrate (Carolina Soil®) (Composition according to the manufacturer: peat, vermiculite, roasted rice husk, limestone, pH: 5.5, electrical conductivity: 0.7 dS m^{-1}, density: 130 kg m^{-3}, water retention capacity: 300% and maximum humidity: 60%) were placed inside the gutters with a spacing of 0.25 m between each other.

A completely randomized design (DIC) with four treatments and eight replications was used. The treatments were: T1 - only freshwater (control); T2 - Thiamine at a concentration of 200 mg L^{-1} (B1); T3 - Niacin at a concentration of 200 mg L^{-1} (B2); T4 - Thiamine and niacin combined, in a concentration of 100 mg L^{-1} of each vitamin (B1 + B2). The application of vitamins was carried out with a manual sprayer on the plants five days after the installation of the pots in the hydroponic system, in a volume of 2 mL of solution per pot. Each experimental plot consisted of a pot containing a lettuce seedling of the cultivar "Pira Roxa" in the first experiment and "Milena" in the second experiment, obtained from a certified nursery. The seedlings of both cultivars had three leaves at the beginning of the experiments.



A - Implementation of Experiment 1; B - Application of treatment with vitamins; C - Harvest of Experiment 1; D - Implementation of Experiment 2; E - Application of treatment with vitamins; F - Harvest of Experiment 2

Figure 1. Average temperature and relative air humidity inside the protected environment during the experiments

In the first experiment (cultivar Pira Roxa), 23 days after the seedlings were transferred to the hydroponic system, intracellular CO_2 concentration (Ci) (μmol CO_2 m^{-2} s^{-1}), transpiration (E) (mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs) (mol H_2O m^{-2} s^{-1}) and CO_2 assimilation rate (A) (μmol CO_2 m^{-2} s^{-1}) were evaluated, These evaluations occurred during the morning (between 8 a.m. and 10 a.m.), when the plants were in full gas exchange activity, using a portable photosynthesis meter (LCi, ADC Bioscientific, Hertfordshire, UK). From the data obtained, the water use efficiency - WUE (A/E) (μmol CO_2 mmol H_2O^{-1}) and instantaneous carboxylation efficiency - CEi (A/Ci) (μmol CO_2 m^{-2} s^{-1}) were estimated.

At 23 and 30 days after the plants were transferred to the hydroponic system, in experiments 1 and 2, respectively, the number of leaves (NL) and the fresh (FW) and dry weight (DW) of plants were evaluated. The plant fresh weight (FW) was obtained by weighing the shoot of the plants (g per plant). The dry weight (DW) was obtained by drying the plant shoot in an oven with forced air circulation at 65 °C until obtaining constant weight (g per plant). Also, the relative chlorophyll content (RCI) was evaluated using a digital chlorophyll meter (CCM-200, Opti-Sciences, Hudson, USA).

Before proceeding with the analysis of variance, the homogeneity of variance and the normality of data were verified using the Cochran and Lilliefors tests, respectively. Then, data were subjected to analysis of variance ($p \leq 0.05$). The means were compared by t-test (LSD) at $p \leq 0.05$. Statistical analyzes were performed using the Sisvar 5.1 software (Ferreira, 2019).

Results and Discussion

Vitamins application influenced Ci, E, A, FW, DW, and NL in experiment 1 and FW, DW, and NL in experiment 2. The gs, WUE, CEi, and RCI in experiment 1 and the RCI in experiment 2 were not affected by the application of the treatments (Table 1).

It was found that the application of vitamins effectively altered the physiological responses of lettuce plants, except for stomatal conductance (Figure 2). In this sense, there was an increase in the intracellular CO_2 content when the vitamins were applied combined (Figure 2A). Also, the joint application of vitamins and the use of niacin alone increased transpiration and CO_2 assimilation rate, with no significant difference for the isolated application of thiamine for this last characteristic (Figures 2B and 2C).

Table 1. Summary of analysis of variance for internal CO_2 concentration (Ci), transpiration (E), stomatal conductance (gs), CO_2 assimilation rate (A), water use efficiency (WUE), instantaneous carboxylation efficiency (CEi) for Experiment 1 and number of leaves (NL), plant fresh weight (FW), plant dry weight (DW), and relative chlorophyll index (RCI), for Experiments 1 and 2, of lettuce plants under hydroponic conditions with the leaf spray application of vitamins.

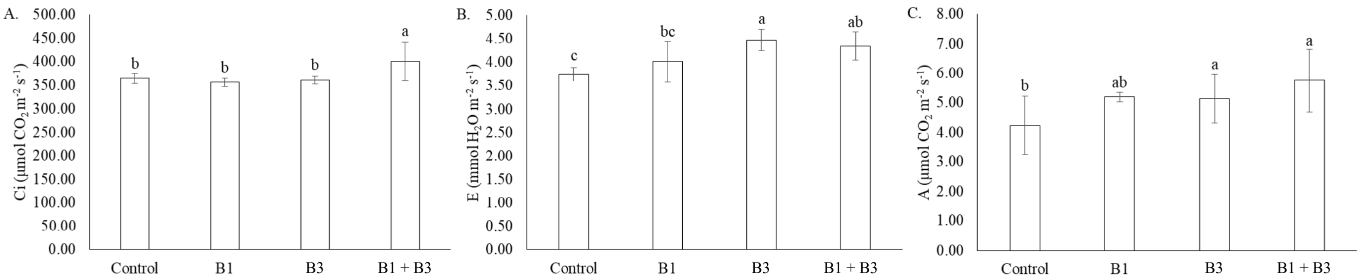
SV	DF	Mean squares				
		Ci	E	gs	A	WUE
		Experiment 1				
Block	3	154.11^{ns}	0.08^{ns}	0.0008^{ns}	0.03^{ns}	0.01^{ns}
Treatments	3	3266.50**	0.88**	0.0014^{ns}	3.16*	0.09^{ns}
Residue	25	530.73	0.09	0.0008	0.78	0.06
CV%		6.21	7.21	12.15	17.37	19.34
		CEi	NL	FW	DW	RCI
Block	3	0.00001^{ns}	0.11^{ns}	81.25^{ns}	0.29^{ns}	10.45^{ns}
Treatments	3	0.00001^{ns}	6.30*	934.66*	3.40*	0.85^{ns}
Residue	25	0.00002	1.35	252.93	0.92	5.37
CV%		35.88	10.63	22.52	22.51	19.31
		NL	FW	DW		RCI
		Experiment 2				
Block	3	1.14^{ns}	152.31^{ns}	0.77^{ns}		0.01^{ns}
Treatments	3	18.49**	3523.58**	12.24*		0.04^{ns}
Residue	25	3.25	402.87	0.92		0.03
CV%		9.69	11.73	14.16		19.31

^{ns} - Not significant ($p > 0.05$), * - Significant at 5% probability level ($p \leq 0.05$), ** - Significant at 1% probability level ($p \leq 0.01$), CV - Coefficient of variation, SV - Source of variation, and DF - degree of freedom

The increase in gas exchange activities may be related, as observed by Ramos et al. (2021), to the increase in stomatal functionality resulting from the application of nicotinamide, the amine form of vitamin B3, and the stomatal density, which was increased from the foliar application of thiamine and the set formed by thiamine and nicotinamide. These increments reflect better stomatal performance, depending on the conditions in which the plants are found (Souza et al., 2010). In this sense, the constant presence of humidity in the system allows high rates of transpiration of plants, an essential factor for the capture of atmospheric CO_2 (Ancu et al., 2014; Thomas et al., 2021) and which started to benefit from the application of vitamins.

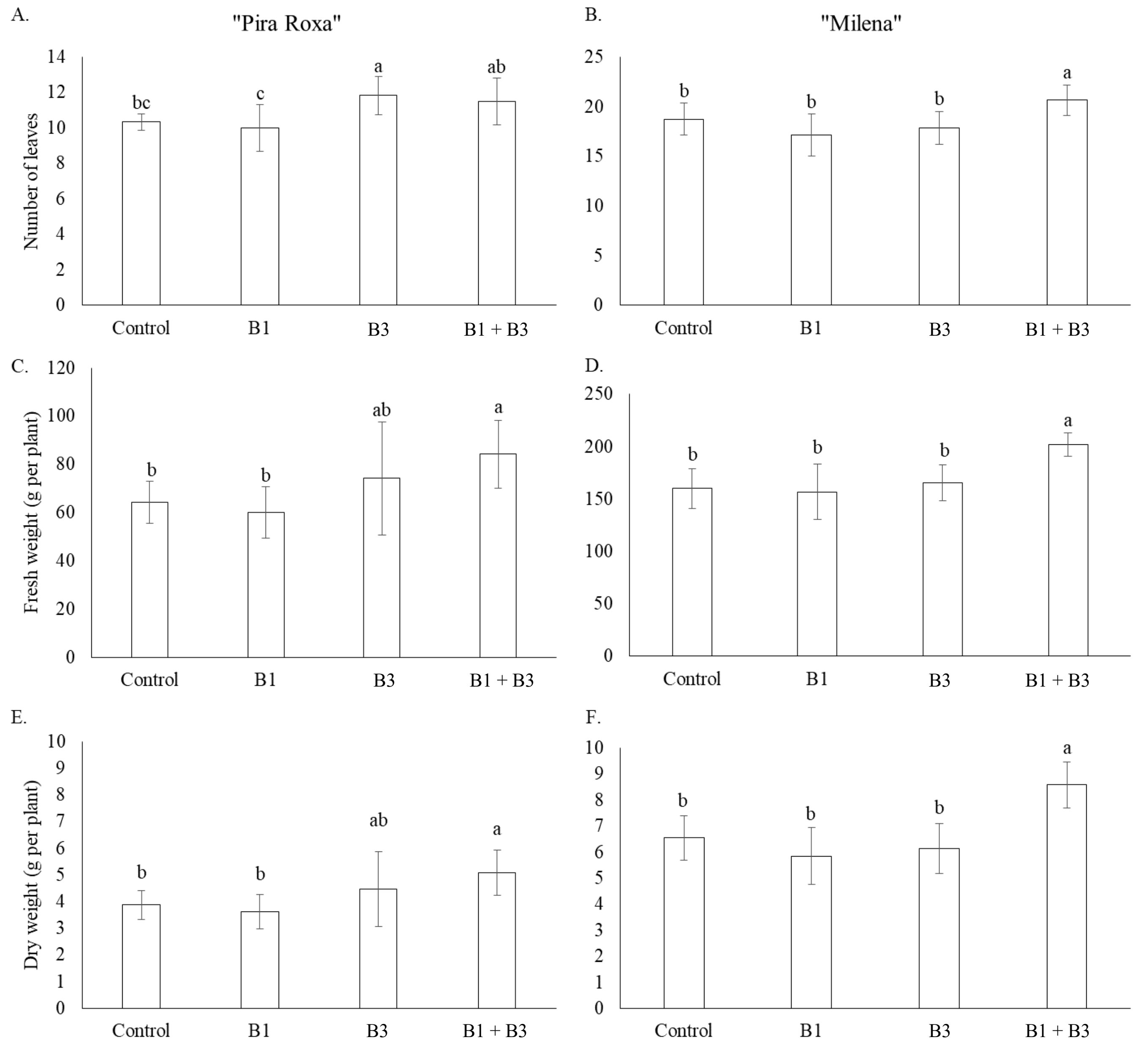
For both cultivars, there was a predominance of the treatment with the combined application of vitamins on the number of leaves and fresh and dry weight of plants, but there was no significant difference for the isolated application of niacin in the cultivar Pira Roxa (Figure 3).

The promotion of development caused by the presence of vitamin B3 in treatments applied to the cultivar Pira Roxa



B1 - Thiamine; B3 - Niacin; B1+B3 - Thiamine + Niacin. Means followed by the same lowercase letter in the column do not indicate a significant difference between them by the t-test (LSD) at $p \leq 0.05$. Bars represent standard deviation (n = 8)

Figure 2. Internal CO_2 concentration - Ci (A), transpiration - E (B), and CO_2 assimilation rate - A (C) of lettuce plants, cultivar Pira Roxa, under hydroponic conditions with the leaf spray application of vitamins



B1 - Thiamine; B3 - Niacin; B1+B3 - Thiamine + Niacin. Means followed by the same lowercase letter in the column do not indicate a significant difference between them by the t-test (LSD) at p ≤ 0.05. Bars represent standard deviation (n = 8)

Figure 3. Number of leaves (A, B), plant fresh weight (C, D), and plant dry weight (E, F) of lettuce cultivars Pira Roxa and Milena under hydroponic conditions with the leaf spray application of vitamins

(Figure 3) is the result of the action of the vitamin on the photosynthetic and respiratory systems, considering that both the promotion of CO_2 assimilation rate and transpiration are directly correlated with fresh and dry weight gain (Table 2). This vitamin acts along the energy transfer sites since this compound is part of nicotinamide adenine dinucleotide (NAD)

Table 2. Estimate of Pearson correlation coefficients between the characteristics of lettuce plants, cultivar Pira Roxa, under hydroponic conditions with the leaf spray application of vitamins

	Ci	E	gs	A	WUE	CEi	FW	RCI	NL	DW
Ci		0.461	0.447	-0.057	-0.302	-0.380	0.075	0.050	0.370	0.075
E			0.911	0.295	-0.216	0.121	0.359	-0.068	0.459	0.359
gs				0.184	-0.289	0.021	0.251	-0.140	0.306	0.251
A					0.865	0.944	0.347	0.181	-0.020	0.347
WUE						0.901	0.165	0.215	-0.257	0.165
CEi							0.292	0.143	-0.133	0.292
FW								0.132	-0.068	0.990
RCI									0.142	0.132
NL										-0.068
DW										
				**	*	*	**			

* - Significant (p ≤ 0.05); ** - Significant (p ≤ 0.01). Blue and orange colors mean positive and negative correlations, respectively. Greater color intensities indicate a greater degree of correlation

and nicotinamide adenine dinucleotide phosphate (NADP) (Kirkland & Meyer-Ficca, 2018).

The synergistic effect caused by the joint use of vitamins on the activity of gas exchange (Figure 2) was also verified in sugarcane plants subjected to water stress during the initial phase of development (Ramos et al., 2021) and on development, growth, and fertility of rice spikelets (Vendruscolo et al., 2019). In addition, the protective effect of this combination was also verified in sweet corn plants subjected to irrigation with saline solution, resulting in lower mass losses (Vendruscolo & Seleguini, 2020). In part, the authors relate the beneficial effects to the action mentioned above of niacin and a protective effect caused by the action of thiamine.

The exogenous application of thiamine increases secondary metabolism compounds; responsible for the plant's defense signals against biotic and abiotic stresses (Goyer, 2010). In this way, the plant is stimulated to produce greater amounts of free proline, reducing the action of oxidative compounds, such as reactive oxygen species, and, consequently, helping to maintain physiological activities, including photosynthesis (Vendruscolo et al., 2022) and also membrane stability (Sayed & Gadallah, 2002). In addition, such signaling still results in the accumulation of energy and nutritional reserves, which can be used during cell division and elongation (Kaya et al., 2015).

However, despite the protective effects of the thiamine application, its presence under controlled conditions of plant growth, such as hydroponic systems, can reduce development (Figure 3). This is because the application of this vitamin results in a priming effect, activating the plant's defense mechanisms against possible stress (Goyer, 2010). In this sense, the application can result in a less efficient process of gas exchange, with slightly lower values of Ci, E, and A, which are directly correlated with the increase in WUE and CEi (Table 2) in response to stomata closure, taking into account the effect of this vitamin on stomatal functionality (Ramos et al., 2021).

In possession of the results, the potential for using niacin alone and its combined application with thiamine was verified. In this sense, the present study brings initial information that can serve as a starting point for future explorations, aiming at the establishment of improved management regarding the use of these compounds in hydroponic cultivation systems.

Conclusions

1. Vitamins affect the morphophysiology of lettuce under hydroponic system conditions.

2. The use of niacin has the potential for use in hydroponic production systems.

3. The combined use of thiamine and niacin provides results superior to those obtained by their isolated application, with a synergistic effect between the compounds being verified.

4. The use of vitamins promotes higher values of CO_2 concentration, transpiration, and CO_2 assimilation rate in the Pira Roxa cultivar and higher values for leaf number and fresh and dry weight in the two cultivars, Pira Roxa and Milena.

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