



SELENIUM IN ACMELLA OLERACEA (L.) R. K. JANSEN GROWN IN HYDROPONICS: THRESHOLDS BETWEEN BIOFORTIFICATION AND TOXICITY

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ABSTRACT

Objective: With the possibility of using non-conventional food plants to minimise the impact of nutritional deficiencies in selenium, this research sought to determine the levels of maximum absorption efficiency and tolerance of *Acmella Oleracea* to the element.

Theoretical background: Almost 1 billion people in the world are deficient in selenium (Se). As it is a fundamental element for human health, vegetable biophotification programmes, as a form of nutritional supplementation, have covered a range of plants such as lettuce, basil, rice, corn, among others.

Method: In a completely randomised experiment, 60 plants were grown in hydroponics, supplemented with Se at doses of 5, 10 and 20 mg.L⁻¹, plus a control. Biometric and physiological variables and Se content in *Acmella Oleracea* were measured. Factor analysis was used to measure the effects of Se and the plant's dose/response relationship.

Results and conclusion: *Acmella Oleracea* grew and assimilated 818.72±58.59 µg.kg⁻¹ in the aerial part when exposed to a dose of 5 mg.L⁻¹ for 28 days, differing positively from the control plants. The maximum dose that the plant tolerated, already showing signs of toxicity, was 10 mg.L⁻¹ for 28 days.

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Research implications: This research has implications for the way *Acmella Oleracea* is managed and can be used as a guide in the breeding process, making this plant an alternative for mitigating Se malnutrition in various parts of the world, especially in the Amazon.

Originality/value: This research is original and in line with the objectives of sustainable development and socio-environmental management, as it shows that the use of unconventional vegetables that are often adapted to various regions of the planet can be a viable alternative to mitigate the various forms of malnutrition and food insecurity.

Keywords: Selenium Absorption, Hormetic Effect, Quantitative Methods, Jambu.

SELÊNIO EM *ACMELLA OLERACEA* (L.) R. K. JANSEN CULTIVADAS EM HIDROPONIA: LIMIARES ENTRE BIOFORTIFICAÇÃO E TOXIDEX

RESUMO

Objetivo: Com a possibilidade do uso de plantas alimentares não convencionais para minimizar os impactos de deficiência nutricional ao selênio, procurou-se nesta pesquisa determinar os níveis de máxima eficiência de absorção e tolerância da *Acmella Oleracea* ao elemento.

Referencial teórico: Quase 1 bilhão de pessoas no mundo apresentam em sua dieta deficiência de selênio (Se). Como é um elemento fundamental à saúde humana, os programas de biofortificação de vegetais, como forma suplementação nutricional, tem abrangido uma gama de plantas como alface, manjerição, arroz, milho, entre outras.

Método: Em experimento inteiramente casualizado, foram cultivadas 60 plantas por hidroponia, suplementadas com Se nas doses de 5, 10 e 20 mg.L⁻¹, mais controle. Foram medidas variáveis biométricas e fisiológicas e o conteúdo de Se na *Acmella Oleracea*. Foi utilizada, a análise fatorial para mensurar os efeitos do Se no jambu e a relação dose/resposta da planta.

Resultados e conclusão: A *Acmella Oleracea* cresceu e assimilou 818.72±58.59 µg.kg⁻¹ na parte aérea, quando exposto a dose de 5 mg.L⁻¹ durante 28 dias, diferindo de forma positiva das plantas controle. A dose máxima que a planta tolerou, já com sinais de toxidez, foi de 10 mg.L⁻¹ durante 28 dias.

Implicações da pesquisa: Esta pesquisa tem implicações na forma de manejo da *Acmella Oleracea*, podendo ser usada como norteadora no processo de melhoramento e tornando esta planta como uma alternativa para mitigar a desnutrição por Se em várias partes do mundo, sobretudo na Amazônia.

Originalidade/valor: Esta pesquisa é original e está alinhada com os objetivos do desenvolvimento sustentável e a gestão socioambiental, pois mostra que o uso de hortaliças não convencionais muitas vezes adaptadas a várias regiões do planeta pode ser uma alternativa viável para mitigar as diversas formas de desnutrição e insegurança alimentar.

Palavras-chave: Absorção de Selênio, Efeito Hormético, Métodos Quantitativos, Jambu.

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1 INTRODUCTION

Hunger in the world has persisted as a complex problem for several centuries, even as the technological advances of recent decades, modern society still faces many challenges in search of food sovereignty and security (Gomes e Medeiros, 2020). Many efforts are being made by UN-linked multilateral organizations such as WFO (*World Food Program*), FAO (*Food and Agriculture Organization*), IFAD (*the International Fund for Agricultural*



Development), among others, which are moving in the right direction towards achieving the goals set by the Sustainable Development Goals (SDGs), especially those aimed at poverty eradication (SDG 1), zero hunger (SDG 2), health and wellbeing (SDG 3). However, in some regions of the world such as Sub-Saharan Africa, Latin America and the Caribbean, for example, the achievement of these goals will still depend on a great effort multilateral bodies, developed and developing nations, as food insecurity has grown over the years in these regions (UN, 2020; Mariutti et al., 2021).

As a developing Latin American country, with large agricultural areas and vast plant diversity, Brazil occupies an important position as a food producer. However, there is great disparity between Brazilian regions in terms of agricultural production and food insecurity problems (Corado et al., 2022; Gomes e Medeiros, 2020). Bezerra et al., (2020) show this disparity, indicating that the northern and northeastern regions of Brazil are those facing the highest levels of food and nutritional insecurity.

The non-use of this vast plant biodiversity as a form of food or even food complementation may be linked to lack of knowledge and exploitation as an alternative to minimize the problem of hunger (Tuler et al., 2019; Silva et al., 2021).

Faced with this problem, the use of non-conventional food plants (NCPs), especially vegetables, can provide various compounds such as beta-carotenes, flavonoids, vitamin C, among others, bringing benefits to human health.

However, it is not only the poor production of food that is plaguing society, but the nutritional quality of these foods. The low concentration or even absence of nutrients such as iron (Fe), zinc (Zn) and selenium (Se) in the population's diet has also become a food insecurity problem (Carvalho et al., 2006). This low concentration of nutrients is more related to the content of these in the soil and their availability to the plants. According to Kabata - Pendias and Szeke (2015) the average Fe content in soils ranges from 3 to 5%, the Zn content from 60 to 89 mg.kg⁻¹ and the Se content ranges from 0.05 to 0.5 mg.kg⁻¹. It can be seen that of the nutrients that have been low in the food, Se is the one with the lowest content in the soil.

Because of this low availability in soils, about 1 billion people do not have Se in their diet (Kieliszek and Sandoval, 2023). This deficiency reflects on health and food safety, since Se is an essential element in humans, helping to reduce the activity of reactive oxygen species, modulation of the immune system, reduction of trace element levels in the human body, and reduce the risk of cancer (Cardoso et al., 2015; Zhang and Song, 2021).

An alternative used to address Se deficiency in the human diet is agronomic biofortification, which consists in improving the nutritional quality of food via nutritional supplementation with the addition of mineral elements such as Se during the plant fertilization process (Khush et al., 2012; Sing et al., 2021).

In this sense, associating biofortification techniques with regional plant species, above all vegetables, can be an important alternative to overcome the deficiency of the element in some areas of Brazil and the world.

Puccinelli et al., (2017) conducted research with biofortified basil with Se in Italy, as a way to bring alternative to that population in the face of nutritional deficiency of the element. Oliveira et al., (2022a) analyzed the biofortification potential of *Acmella Oleracea* vegetable (L.) R.K. Jansen and reported that supplementation of 3 mg.L⁻¹ Se in the form of sodium selenate in hydroponia produced a plant with the ability to concentrate on its edible parts approximately 50 µg of Se.

The jambu (*Acmella Oleracea* L.R.K. Jansen) is a vegetable of great gastronomic and cultural value in the state of Pará and that in recent years has gained importance for the pharmaceutical and cosmetics industry from studies that have proven the potential of bioactive



substances such as espilantol present in its leaves, stalks and inflorescences (Barbosa et al., 2016; Uthpala and Navaratne, 2020).

The plant has characteristics that allow at the same time to contribute to the food security of riverside dwellers, as well as signifying an important source of income for small producers in the municipalities of the State of Pará (Homma et al., 2014)

These characteristics mean that the vegetable has a strategic importance in the cultivation, food security and industrialization of the main active ingredients of the Amazon natural resources, and can be developed in the form of sustainable models that guarantee productivity, financial return and preservation of the culture and food security in the Amazon region, but for this scenario to be confirmed, it is fundamental to develop techniques that optimize production and guarantee higher quality in production.

However, some elements that are even beneficial, depending on the dosage used, can bring toxic effects to the plant and to humans. For most cultivars of agronomic interest this threshold between nutritional enrichment of the plant and toxicity is already established, for example lettuce, basil, rice, corn, among others. However, for regional cultivars such as *Acmella Oleracea*, experiments of this nature have not yet been conducted. According to the *Food Nutrition Board (Institute of Medicine, 2000)*, the recommended daily dose of Se is $55 \mu\text{g}.\text{day}^{-1}$ and the tolerable dose is $400 \mu\text{g}.\text{day}^{-1}$ of Se, these being the limits that should be followed for biofortification.

Another point to be highlighted is the form of analysis of experimental data. It is necessary that the analysis of data does not consider the study of a variable in isolation, without establishing the appropriate correlations between the variables. In agricultural experimentation, the dose levels of a given element will influence for example growth, mass gain, CO_2 assimilation rate, among other variables. These relationships and similarities should be highlighted in the light of a methodology that manages to translate the interaction between the measured variables in a more synergistic manner. In this sense, the use of multivariate methods such as factor analysis brings the ability to establish linear relationships of the original variables, reducing them to a smaller number of uncorrelated latent variables, with high explanatory power and ability to observe several characteristics of the phenomenon studied simultaneously (Hair et al., 2009; Silva, 2016; Leal, et al., 2021; Jena et al., 2023).

This panorama gives rise to the following question: what is the dose limit between biofortification and selenium toxicity that does not jeopardize the development of *Acmella Oleracea* and neither human health? Which multivariate method is most suitable for dose/response agronomic experiments? Therefore, the objective of the present study was to analyze the effects of Se concentration in *Acmella Oleracea* and to determine, through multivariate analysis, the tolerance levels of the plant to the element.

2 MATERIAL AND METHODS

The experiment was conducted in the months of August and September 2019, in the vegetation house of the Institute of Agrarian Sciences of the Federal Rural University of Amazonia ($1^\circ 27' 13.70''$ South and $48^\circ 26' 32.65''$ West of Greenwich). With an altitude of around 14 meters, the area where the experiment was developed has a hot and humid equatorial climate, Af category of Koppen (Kottek et al., 2006).

The cultivation system was hydroponic in 1.5L vessels. The nutrient solution used was that of Hoagland and Arnon (1950). The vessels were filled with inert substrate (sterile ground silica), coated with aluminum foil to avoid the incidence of solar radiation and perforated at the base, with the introduction of a silicone tube to facilitate daily recirculation of the solution.



The seeds of *Acmella Oleracea*, harvested at UFRA's seed bank, were sown on August 3, 2019 in polystyrene trays with 128 cells filled with organic compound. In each cell, 10 to 15 seeds of the regional variety *Acmella Oleracea* yellow flower were placed. After 10 days of germination, thinning was done, leaving one seedling per cell.

After twenty-two days, the seedlings with four expanded leaves were removed from the polystyrene tray, their roots were washed with distilled water and then transplanted to the silica containing vessels and 50% of the ionic strength to facilitate adaptation, thus remaining for seven days. After the seven days of acclimatization, the nutritional solution had its ionic strength increased to 100%. The nutrient solution was composed of 1 mL.L⁻¹ NH₄NO₃, 4 mL.L⁻¹ de KNO₃, 5 mL.L⁻¹ de Ca(NO₃)₂, 2 mL.L⁻¹ de MgSO₄, 1 mL.L⁻¹ de Fe-EDDHA (21.6 g.L⁻¹ de EDDHA, 286 mL.L⁻¹ de 1M KOH e 24.9 g.L⁻¹ de FeSO₄.7H₂O) and 1 mL.L⁻¹ of micronutrient solution composed of 2.86 g.L⁻¹ de H₃BO₃, 1.81 g.L⁻¹ of MnCl₂.4H₂O, 0.22 g.L⁻¹ de ZnSO₄.5H₂O, 0.08 g.L⁻¹ de CuSO₄.5H₂O, 0.02g.L⁻¹ de H₂MoO₄.

Daily, during the whole experiment, the pH and the electrical conductivity of the nutrient solution were monitored. The pH was maintained in the range of 5.5, at 6.5, and the electrical conductivity in the range of 1.0 - 1.8 µS.cm⁻¹.

The experimental design was entirely casualized, with four treatments, roughly fifteen repetitions per treatment, making a total of 60 plants, one plant per vessel. Three doses of selenium were used (5, 10 and 20 mg.L⁻¹), applied in the form of sodium selenate (Na₂SeO₄) and plus control (Puccineli, et al., 2017; Oliveira et al., 2022a).

Three harvests were made throughout the plant's vegetative cycle at 14, 21 and 28 days after transplantation (DAT). At each stage five plants were harvested by treatment and after each harvest, the selenium-enriched nutrient solution was renewed (Puccineli, et al., 2017; Oliveira et al., 2022).

2.1 Biometric Analysis

After each harvest, the following biometric variables were measured: (i) Plant Height (AP) - using a millimeter ruler, measuring from the soil surface to the apex of the main stock; (ii) Stem Diameter (DC) - using a pachimeter, measuring the stem at 0.5 cm from the substrate; (iii) Number of Leaves (NF) - counting of the total leaves emitted by the plant; Number of Branches (NR) - counting of total branches emitted by the plant; Dry Mass of the Air Part (MSPA) - weighing of the analytical leaves and stems precision method after drying at 65 °C in a forced circulation oven until constant weight is reached; Dry Root Mass (MSR) - weighing of the plant root in an analytical precision balance soon.

2.2 Determination of Gaseous Exchanges

The gas exchange variables were measured on the first expanded leaf from the apex to the base of the plant. The plants were evaluated at 14, 21 and 28 DAT. The net assimilation rate of CO₂ (A), the stomatic conductance to water vapor (g_s), the perspiration rate (E) and the internal CO₂ concentration (C_i), were measured between 11:00 and 13:00 using a portable open flow gas exchange system (LI-6400XT, LI-COR, Lincoln, NE) under an external CO₂ concentration of 400 µmol mol⁻¹ air and artificial PAR of 1 1,000 µmol m⁻² photons, according to Castro et al. (2017) and Sampaio et al. (2018).

The measurement range was adjusted by obtaining a diurnal curve of gas exchange for the *Acmella Oleracea* in the area where the study was conducted.



2.3 Chemical Analysis

Root, stem and leaf samples from *Acmella Oleracea* after circulating greenhouse droughts 65°C have been ground. After grinding the samples, an aliquot of 0,5 g was removed and digested in an Ethos Easy microwave oven with 5,0 ml of HNO₃, 1 ml of H₂O₂ (30% mm-14) and 2 ml of ultrapure water. The microwave digestion ramp consisted of three steps: temperature rise from 0 to 180°C in 10 minutes at 1800 w, temperature stay of 180°C for 20 minutes and ventilation for 50 minutes (Oliveira et al., 2022a).

Determination of Se concentration in plant parts was determined by atomic absorption spectrometry with Varian AA 240 Z graphite furnace with GTA 120 graphite tube atomizer and Zeeman background corrector (Varian, 1988). A hollow cathode lamp was used for the determination of Se at a wavelength of 196 nm, with palladium solution as the modifier matrix. The dragging gas was analytic argon 5.0, with pyrolysis temperature of 120°C, atomization of 1000°C and cleanup of 2600°C (Zangmo and Siripinyanond, 2019). All the analyzes were carried out at the Amazonian Water Quality Laboratory of the State University of Pará (UEPA).

2.4 Multivariate Analysis

After the laboratory analyzes, the results were placed in a matrix form, containing in the first column the doses/days of exposure and in the rows the variables analyzed.

The relationship between the variables was measured through Factorial Analysis (AF), with extraction by main components, as this transforms a large set of variables into a reduced number of factors, bringing in a clear explanation of the relation of the original variables (Hongyu et al., 2015). To achieve a better distinction between factors, the factors were rotated by the Varimax method after extraction to maximize the factor loads within each factor.

In this way the factors (latent variables) have uncorrelated linear combinations of the original variables n (plant parameters) and m observations (samples), $n < m$, explaining decreasing maximum proportions of the variation of the original data (Ribas e Vieira, 2011; 2013; Hougyu et al., 2015; Field, 2020). The factorial analysis model gives the following expression (equation 1):

$$X_i = a_{11}f_1 + a_{12}f_2 + \dots + a_{im}f_m + d_iu_i, \quad (1)$$

Where:

x_i = normalized variable with $i = 1, 2, \dots, n$ (variables = plant parameters) and $j = 1, 2, \dots, m$ (observations = samples); a_{ij} = represents the factor loads of the m -th factor for the i -th variable; f_m = represents the common factor value m -th for the j -th observation; d_iu_i = residue.

For the determination of the factorial scores (relation between the factorial loads and the observations), the value of the variable i was multiplied by the coefficient of the corresponding factorial score, according to equation 2.

$$E_j = \sum b_{ji}X_i = b_{j1}X_1 + b_{j2}X_2 + \dots + b_{jp}X_p \quad (2)$$

Where:

E_j are the factorial scores, $b_{1,\dots,p}$, are the coefficients of the scores and p is the number of variables. Scores were used to define the level of influence of Se doses in relation to the days of exposure of the plant to the element (Cunha, et al., 2008; Hair et al., 2009; Oliveira et al., 2022 a, b).



In order to meet the AF assumptions, the sample adequacy measurement tests (*Kaiser-Meyer-Olkin of Sampling Adequacy* - KMO) that should be higher than 0.7 and the Bartlett sphericity test that verifies the absence of relationship between the observed variables (Hair, et al., 2009; Ribas and Vieira, 2011; Matos and Rodrigues, 2019) were applied.

The software used to perform the factorial analysis was R version 4.2.3, through the packages "psych" and "metan" (R Core Team, 2020; Olivoo and Lucio, 2020).

Subsequently, the extraction of the factors and the obtaining of the factorial scores, it was possible to estimate the influence of Se on the biometric and physiological variables. Factor 1-related factor scores thus captured the influence of Se on plant height (AP), stem diameter (DC), number of leaves (NF), number of branches (NR), air dry mass (MSPA) and root dry mass (MSR), which report how the element influenced plant growth and mass gain (biometric variables). Factor 2-related factorial scores captured the influence of Se on the net assimilation rate of CO₂ (A), stomatic conductance (gs), perspiration (E) and internal CO₂ concentration (Ci), outlining how Se influenced the photosynthetic apparatus of the *Acmella Oleracea* (physiological variables). Thus, the influence of the Se on biometric and physiological variables can be better explained by constructing the indicators: biometric (related to biometric variables) and physiological (relates to physiological variables), according to equation 3.

$$In_i = \sum_{j=1}^n \frac{\lambda_j}{\sum \lambda_j} F_{ij}^* \quad (3)$$

Where:

In_i is the constructed indicator for each factor, j is the j -th characteristic root, n is the number of factors extracted in the analysis, F_{ji}^* is the j th-th factorial score of the i th plant and $\sum \lambda_j$ is the sum of the roots referring to the expressed factors. To measure the relative share of factor j in the explanation of the total variance captured, the weighted average of the roots was used $\lambda_{\theta} = \lambda_j / \sum \lambda_j$ (Cunha et al., 2008; Oliveira et al., 2022a and b).

To facilitate the analysis of variance and the construction of the regression model of the indicators, the values of the factorial scores (F_{ji}^*) were transformed into positive or equal to zero, being placed in the first quadrant, according to equation (4), proposed by Lemos (2001).

$$F_{ji}^* = \frac{F_{ji} - F_j^{\min}}{F_i^{\max} - F_j^{\min}} \quad (4)$$

The indicators were submitted to regression analysis to determine the maximum point of influence of Se on *Acmella Oleracea* and obtain the doses of maximum growth, mass gain and increase of the plant's photosynthetic capacity.

Se content data (aerial and root part) as well as multivariate indicators were submitted to variance analysis (ANOVA) and the means were compared by Scott-Knott test (SK), $p < 0.05$, with the use of R software version 4.2.3, easyanova package (R CORE TEAM, 2020).



3 RESULTS AND DISCUSSIONS

The Se concentration in the aerial part and root of *Acmella Oleracea* are shown in Table 1 and express how the element was captured and distributed in the plant throughout the experiment. The increased supply of Se in nutrient solution promoted greater absorption of the element in the aerial part and roots of the plant throughout the experiment.

With 14 exposure days, a significant increase in the Se concentration in the aerial part occurred, with a maximum value of $318.02 \pm 17.56 \mu\text{g.kg}^{-1}$ in plants supplemented with 20 mg.L^{-1} of Se and a minimum of $114.40 \pm 11.34 \mu\text{g.kg}^{-1}$ in plants supplemented with 5 mg.L^{-1} , demonstrating a linear trend of assimilation of Se described by equation ($y = 103.31x + 10.62$; $R^2 = 0.9982$).

Table 1 - Mean Se concentration in *Acmella Oleracea* cultivated in hydroponia

Days of exposure	Se doses in mg.L^{-1}	Se content in $\mu\text{g.kg}^{-1}$	
		Airside	Root
14	5.00	$111.40 \pm 11.34 \text{ cC}$	$19.19 \pm 2.25 \text{ cC}$
14	10.00	$222.31 \pm 28.72 \text{ bC}$	$39.78 \pm 5.44 \text{ bC}$
14	20.00	$318.02 \pm 17.56 \text{ aB}$	$70.33 \pm 6.37 \text{ aC}$
21	5.00	$455.64 \pm 71.08 \text{ cB}$	$61.41 \pm 7.21 \text{ cB}$
21	10.00	$603.79 \pm 61.33 \text{ bB}$	$96.39 \pm 8.36 \text{ bB}$
21	20.00	$866.62 \pm 72.02 \text{ aA}$	$145.61 \pm 19.31 \text{ aB}$
28	5.00	$818.72 \pm 58.59 \text{ aA}$	$127.66 \pm 16.20 \text{ bA}$
28	10.00	$974.71 \pm 194.3 \text{ aA}$	$133.59 \pm 15.24 \text{ bA}$
28	20.00	$790.45 \pm 40.44 \text{ aA}$	$180.20 \pm 26.05 \text{ aA}$

The lower case letters point to significant differences between Se doses in the same period (days) of exposure; the upper case letters point to significant differences between exposure days for the same Se dose, according to the Scott-Knott test ($p < 0.05$).

Source: Prepared by the authors, 2024.

The 21-day SE absorption model also showed a linear trend ($y = 27,239x + 324.22$; $R^2 = 0.8847$) at the 20 mg.L^{-1} dose inducing absorption of $866.62 \pm 872.024 \mu\text{g.kg}$, Se-1 in the air. The data presented in table 1 show that with 28 exposure days there was no significant difference in the content of Se in the aerial part as the doses were increased, indicating that the increase of Se in the solution did not provoke a response in the translocation of the same to the aerial part of the plant.

The plants supplemented with 10 mg.L^{-1} of Se and 28 days of exposure were those that showed the highest content of the element in its aerial part ($974.71 \pm 194.3 \mu\text{g.kg}^{-1}$), and from these data, the limit of exposure time and concentration of Se should be established that is *Acmella Oleracea* can stay exposed.

According to Guinardi and Schiavon (2017), the uptake of Se is linked to direct competition by membrane transporters, through the expression of some sulfate-transporting genes such as SULTR1;1 and SULTR 1;2 both with high affinity to Se, which would justify the significant increase of Se in the aerial part of *Acmella Oleracea* with the increase of the element in the nutritive solution. However, as shown in table 1, the 28-day exposure showed no significant difference in the absorption of Se in the aerial part of the plant, indicating that with the passing of days, there was a reduction in the absorption of the element that potentially occurred due to exceeding the tolerance limit of the plant.

Se concentrations found in the root of the plant show the same tendency of assimilation by the aerial part, evidencing the capacity of *Acmella Oleracea* to partition the ^{element}. It should be noted that with 28 days only the plants exposed to the dose of 20 mg.L^{-1} showed significant difference, with content of $180.20 \mu\text{g.kg}^{-1}$.



In this sense figure 1 shows the visual effects of toxicity to the Se, with reduced plant size, chlorosis, and necrosis of leaves and roots when exposed to the highest doses (20 mg.L^{-1}) of Se and more exposure days. It can be noticed that with 14 days of exposure there is already a reduction in the size of the plants exposed to 10 and 20 mg.L^{-1} of Se in relation to the control plant. This phenomenon worsened as exposure time increased.

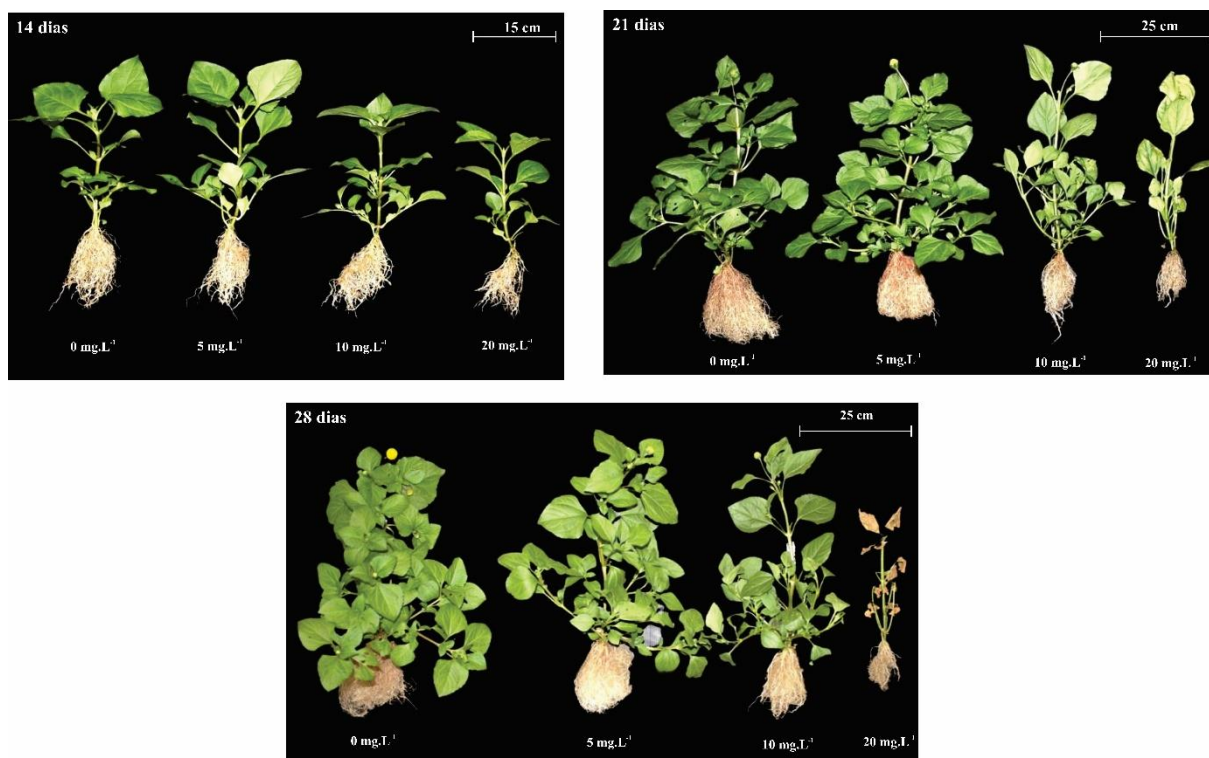


Figure 1 - Toxicity symptoms of *Acmella Oleracea* exposed to increasing doses of Se in nutritive solution
Source: Prepared by the authors, 2024.

Sun et al., (2023), when studying the effect of melatonin synthesis, vegetative growth and sugar content in cherries with applications of Se doses, highlight that the variables analyzed increased and then decreased with the increase of Se concentration, indicating that the cherry showed toxicity with the increase of doses. Kolbert et al., (2019) show that the increase of Se in plants causes the element to be incorporated into amino acids and proteins, in the process of replacing sulfur (S), giving rise to poorly formed selenoproteins considered toxic, an effect also observed in the data of this study, which show the beneficial and toxic effects of Se in *Acmella Oleracea* with increasing doses of the element.

3.1 Multivariate Data Analysis

All 10 variables analyzed proved to be significant in the definition of the factorial model. The construction of the correlation matrix (Figure 2) made it possible to identify the association of variables, highlighting the general trend of the data. The strong positive correlation is observed between all biometric variables, among them are: MSPA and NF ($r=0.93$), NF and NR ($r=0.91$), AP and NR ($r=0.90$). The physiological variables also showed strong positive correlation among themselves, highlighting the correction between g_s and E ($\text{DAR}=0.86$). The correlations between the biometric and physiological variables were weak and negative, not showing a direct relationship *between these variables under the influence of the Se in the plant*.



The C_i was the variable that showed weak positive correlation with the other physiological variables (A , g_s and E) and not significant with the biometric variables (Figure 2)

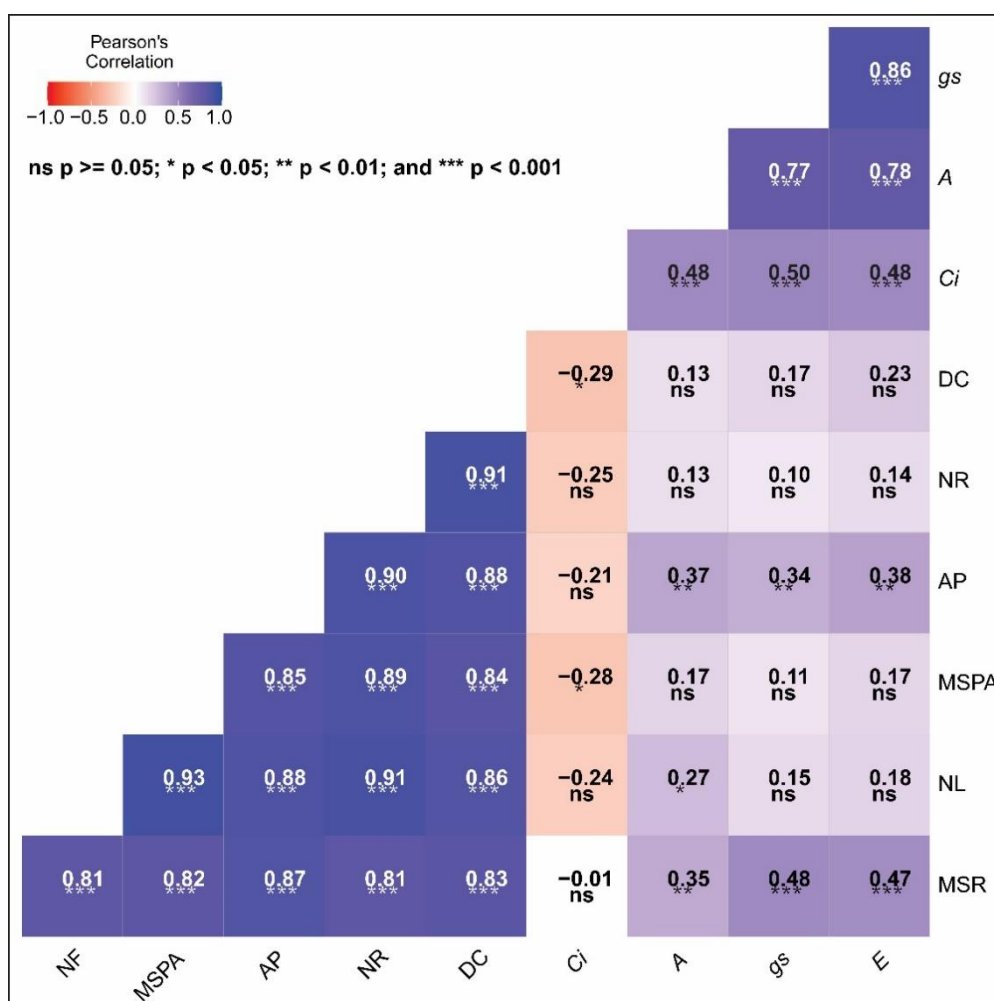


Figure 2 - Correlation matrix between biometric and physiological variables of *Acmella Oleracea* exposed to increasing doses.

NF: Number of leaves; NB: Number of branches; DC: Stem diameter (mm); PA: Plant height (cm); MSR: Dry root mass ($\text{g} \cdot \text{plant}^{-1}$); MSPA: Dry mass of the upper part ($\text{g} \cdot \text{plant}^{-1}$); A : CO_2 assimilation rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$); g_s : Stomatic conductance ($\text{mol H}_2\text{O m}^{-2} \cdot \text{rail} \cdot \text{s}^{-1}$); E : Transpiration ($\text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$); C_i : Internal carbon concentration ($\text{mmol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$). (ns=not significant).

Source: Prepared by the authors, 2024.

The next step to factor analysis was the decomposition of the correlation matrix to reduce the dimension of interrelated variables into smaller dimension. The matrix was extracted and rotated by the varimax method to capture the best distribution of the original variance. The new latent variables were formed by common and independent (uncorrelated) factors. The model that best fitted the data was composed of 2 factors with an explanation of 85.83% of the total variance of the original data according to Hair et al., (2009) and presented in table 2.

The first factor (F1), explained 54.72% of the data variability and is associated with biometric variables, grouping most of them. Of the original descriptors the ones that show higher load in the first factor were: number of branches (NR), stem diameter (DC), number of leaves (NF), dry mass of the air part (MSPA), in contrast to dry mass of root (MSR), it was the one that showed lower load for the first factor (Table 2). It is evident that the First factor (F1),



explains the growth and gain of the mass of the plant exposed to Se, since it was able to capture the information of the biometric variables in a new latent variable (F1).

The second factor (F2), explained 31.06% of the variability of the data associating the physiological variables: CO₂ assimilation rate (*A*), Stomatic conductance (*g_s*), Transpiration (*E*) and Internal carbon concentration (*C_i*). For the second factor, perspiration was the variable that showed higher factorial load and internal carbon (*C_i*) that showed lower factorial load (Table 2). In this case the second factor (F2), explained how the plant's photosynthetic apparatus behaved in relation to the Se doses. The commonality between the variables also presented high values, showing the high correlation between them, highlighting the *C_i* that presented the lowest commonality among the variables that describe the factor 2 (Table 2).

Table 2 - Matrix of Factorial Loads (*Loadings*) and Communalities (*h²*)

Variables	Factorial Loads		Communality	MSA
	F1	F2		
Number of branches (NR)	0.96	-0.03	0.92	0,84
Number of sheets (NF)	0.95	0.04	0.91	0,83
Aerial dry weight (MSPA)	0.95	-0.01	0.90	0,82
Stem diameter (DC)	0.94	0,01	0.89	0,89
Height of plant (AP)	0.94	0.20	0.93	0,86
Dry root mass (MSR)	0,88	0.34	0.88	0,82
Stomatic conductance (<i>g_s</i>)	0.16	0,92	0.87	0.75
Carriage (<i>E</i>)	0.20	0,91	0.86	0.78
Net assimilation rate of CO ₂ (<i>A</i>)	0.41	0,87	0.79	0.63
Internal CO ₂ concentration (<i>C_i</i>)	-0.33	0,72	0.63	0.59
Characteristic Root λ_1	5,477	3.06		
Proportion of variance explained (%)	54,772	31,062		

KMO = 0.814; Bartlett Test - (X^2) = 772.828, significance at 1%; RMSR = 0.040; MSA (sample adequacy measure). Determination of the number of factors by the eigenvalue criterion. Factor extraction by main components with varimax orthogonal rotation.

Source: Prepared by the authors, 2024.

To evaluate how the increase in doses and exposure days influenced the biometric and physiological variables, we analyzed the means of Factor Scores (EF), because these represent the 60 plants observed in the experiment and relate to the factors of the latent variables (Table 3).

The dose of 5 mg.L⁻¹ showed a significant difference in the three exposure periods, both in the EF1 (biometric) score and in the EF2 (physiological) score, showing a beneficial effect of Se on *Acmella Oleracea* throughout the experiment. This effect may be related to increased antioxidant activities of Se, reducing the effects of harmful substances that can cause stress in the plant with production of reactive oxygen species (Broadley et al., 2012; White, 2016; Oliveira et al., 2022b; Tufail. et al., 2023). It is noted that the EF2 (physiological) Score presented a greater factorial load than the EF1 (biometric) Score in all doses with 14 days of experiment. This fact may be related to the *Acmella Oleracea* vegetative cycle, where the plant's photosynthetic apparatus is being more demanded, occurring in the first weeks of cultivation. In the last two weeks (21 and 28 exposure days), a reversal in factor scores is observed, with EF1 showing higher value at all doses of Se, compared to EF2, indicating a loss of photosynthetic efficiency and increase in growth (Sampaio et al., 2021; Oliveira et al., 2022a, b).

With 28 days of experiment there was a strong reduction in values of the EF1 and EF2 Factor Scores at doses of 10 and 20 mg.L⁻¹, indicating the toxicity of *Acmella Oleracea* to the element. Oliveira et al., (2022b) when studying the behavior of *Acmella Oleracea* exposed to Cd show that small doses of the metal (3 mg.L⁻¹ Cd) accelerated the rate of photosynthetic



assimilation of CO₂, stomatic conductance, transpiration and increase in growth and mass, but with the increase in Cd concentration, there was a reduction in photosynthetic activity and consequently affecting its development. Also according to Oliveira et al., (2022b), this binary behavior of *Acmella Oleracea* is called the hormetic effect, described also by Calabrese and Daldwin (2003) and Calabrese and Blain (2009) as a response that some organisms, in this case plants, present to a stress agent, in which this agent induces, in small concentrations, the increase of the plant's metabolic activity and in higher concentrations the plant will present symptoms of toxicity, similar behavior that *Acmella Oleracea* presented in this study in relation to the SE.

Table 3 - Matrix of the mean of the Factor Scores (EF) of *Acmella Oleracea* plants exposed to Se in hydroponia.

Days of exposure	Se doses in mg.L ⁻¹	EF1 (biometric)	EF2 (Physiological)
14	0.00	0.8526 bC	1.4913 b B
14	5.00	0.9812 BC	1.6397 to A
14	10.00	0.8714 bC	1.4910 b B
14	20.00	0.4831 cC	1.3514 c A
CV (%)		7.01	4.51
21	0.00	1.6746 bB	1.6903 to A
21	5.00	1.9344 aB	1.8155 to A
21	10.00	1.5524 bB	1.6719 to A
21	20.00	0.8176 cB	1.2150 b B
CV (%)		7.93	9.11
28	0.00	2.1609 bA	1.0517 b C
28	5.00	2.5732 aA	1.1950 to B
28	10.00	1.9224 cA	1.1604 to C
28	20.00	0.9732 dA	0.5010 c C
CV (%)		4.70	8.42

The lower case letters point to significant differences between Se doses in the same exposure period (days); the upper case letters point to significant differences between exposure days for the same Se dose according to the Scott-Knott test ($p < 0.05$).

Source: Prepared by the authors, 2024.

Thus the binary behavior of Se in *Acmella Oleracea*, as in other plants, is related to the availability and concentration of the element in its growth medium. According to Broadley et al., (2012), Gupta and Gupta (2017) and White (2016) if available as selenate (SeO₄⁻²), it uses the transporters and sulfate channels (SO₄⁻²), present in the plasma membrane of the roots, as an absorption medium. Under high concentration of sulfate, the selectivity of transporters induces a higher absorption of S and reduces the effectiveness of Se uptake, however as the Se increases in the solution, it will cause inhibition of S uptake and synthesis of sulfur compounds in the plant, and may result in the expression of nutritional disorders

Geoffroy et al., (2007), assert that Se can take the place of sulfur in different Fe-S compounds. According to these authors, chloroplasts host several Fe-S proteins involved in photosynthesis. Due to the chemical similarity between Se and S, in an environment of high Se concentration, Se is metabolized along the same S pathways, forming selenoamino acids that are incorporated into proteins, thus reducing the photosynthetic activity of the plant exposed to high doses of the element.

Adebayo et al., (2019) highlight that increasing Se concentration in plant roots above the tolerable limit causes lesions, growth retardation, chlorosis, wilting and leaf drying, because the element, in plants not tolerant to high doses, induces oxidative stress and malformation of selenoproteins, reducing the production of cysteines by the competition of Se with S and thus damaging the protein chain of plants not tolerant to the element, compromising their growth, as demonstrated in this study with *Acmella Oleracea*.



Through the 1st derivative of the multivariate indicator regression models, it was possible to estimate the maximum beneficial effect dose of Se in *Acmella Oleracea* (Table 4).

Table 4 - Regression models of biometric and physiological indicators containing estimates of doses of maximum beneficial effect stimulated by the Se in *Acmella Oleracea* cultivated in hydroponia.

Indicator	Days of exposure	Equation	Dose Estimated	R ²	p-Value
Biometric	14	$Y = -0.002284x^{2**} + 0.02621x^{***} + 0.8675^{**}$	5.74	0.9159	<0,0001
	21	$Y = -0.003951x^{2***} + 0.03262^{xns} + 1.7279^{***}$	4.12	0.8725	<0,0001
	28	$Y = -0.005163x^{2***} + 0.03743^{xns} + 2.2575^{***}$	3.62	0.8882	<0,0001
Physiological	14	$Y = -0.001180x^{2**} + 0.01471^{xns} + 1.597^{***}$	6.23	0.5409	0,0005
	21	$Y = -0.002482x^{2**} + 0.02472^{xns} + 1.7076^{**}$	4.97	0.7359	<0,0001
	28	$Y = -0.003814x^{2***} + 0.04885x^{***} + 0.10501^{***}$	6.40	0.9298	<0,0001

*** significant 1% probability; ** significant at 5% probability; ns - non-significant (F-test); x=dose; x² = squared dose.

Source: Prepared by the authors, 2024.

Regarding plant growth, the biometric indicator shows that during the first 14 exposure days the dose estimate to induce maximum growth of *Acmella Oleracea* was 5.74 mg.L⁻¹ of Se. At 21 days the maximum growth dose decreased to 4.12 mg.L⁻¹ of Se and further decreased with 28 exposure days to 3.62 mg.L⁻¹ of Se. It can therefore be inferred that the increased exposure time of *Acmella Oleracea* to Se caused the reduction of plant tolerance. This same tendency is demonstrated by the physiological indicator (Table 4).

In general, the plant's behavior towards Se showed a polynomial tendency with maximum growth and photosynthetic activity at concentrations ranging from 3.63 to 6.40 mg.L⁻¹ of Se in nutritional solution.

4 CONCLUSIONS

The dose of 5 mg.L⁻¹ in the nutrient solution induced a beneficial effect on *Acmella Oleracea*¹, leading to increased physiological performance, growth and mass gain. With 28 days of exposure, the Se concentration in the aerial part of the plant exposed to 5 mg.L⁻¹ was 818.72±58.59 µg.kg⁻¹. *Acmella Oleracea* showed low tolerance to the element as demonstrated by the presence of chlorosis in the leaves, reduction of the mass of the upper part and root in the plants exposed to the dose of 10 mg.L⁻¹, as well as necrosis of the leaves and roots of the plants exposed to 20 mg.L⁻¹.

Because of the way Se is absorbed by plants, the toxicity to the element may be related to competition in the absorption and substitution of S for Se in cysteines and methionines, and may cause inhibition of sulfur compounds in *Acmella Oleracea*.

The use of a biofortified regional vegetable can be an alternative for the food security of the Amazon population, when it is a way of adding value to a product that is easy to grow and widely distributed in the north of Brazil.

The use of multivariate indicators was adequate to capture in an integrated manner the effect of each explanatory variable described in this study. Because through these indicators it was possible to understand in a systemic way the relationship between the doses of Se given in the solution and the behavior of *Acmella Oleracea*. Especially with the possibility of reducing the large number of descriptor variables of the plant's behavior in a far smaller number of indicators, thus facilitating the understanding of the cause and effect relationship of Se in *Acmella Oleracea*.



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