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ALLIUM CEPA SEEDLINGS RESPONSE TO STRESS GENERATED BY CHEMICALS

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Abstract

This research analyzed the response induced by certain chemicals in Allium cepa seedlings under laboratory conditions, University of Life Sciences "King Mihai I" from Timişoara. Onion bulbs with roots were treated with water (V1), copper sulfate ($CuSO_4$ 300 mg L^{-1} – V2), Faster insecticide (0.3 ml L^{-1} – V3, 0.4 ml L^{-1} – V4, 0.5 ml L^{-1} – V5) and Karate zeon insecticide (0.15 mg L^{-1} – V6; 0.20 mg L^{-1} – V7; 0.25 mg L^{-1} – V8). Morphological parameters (root length, RL; leaf number, LN; leaf length, LL) were determined. The results of the experimental trials presented statistical safety ($p<0.001$). For RL morphological parameter, significant differences from the mean ($\overline{RL}$ = 4.62 cm) were shown by the variants V1 (ΔRL = 4.09 cm; $p<0.01$), and variant V2 (ΔRL = -3.32 cm; $p<0.01$). In the case of the LN parameter, significant differences compared to the mean ($\overline{LN}$ = 0.99) were presented by variant V1 (ΔLN = 0.91, $p<0.001$), and variants V2 and V3 (ΔLN = -0.55, ΔLN = -0.50, $p<0.05$). For LL morphological parameter, compared to the mean ($\overline{LL}$ = 4.41), significant differences were presented by variant V1 (ΔLL = 6.07, $p<0.001$), and variants V2 and V3 (ΔLL = -3.39, ΔLL = -2.77, $p<0.05$).

Key words: *Allium cepa, pesticides, morphological indices, root system, seedlings, stress response.*

INTRODUCTION

Vegetables are important in human nutrition due to their fiber, nutrient content and a wide range of active principles (Bassey et al., 2021; Barbu et al., 2022; Drăghici et al., 2023).
The root system of vegetable plants is diverse in typology, in relation to the plant species and specific influencing factors, and is important for the relationship of plants with the soil, or growing substrate, for the absorption of water and nutrients (Nziengui & Ditengou, 2023).
Vegetable plants develop specific activities (enzymatic, microbiological, ion exchange, etc.) at the level of the root system (root hairs, rhizosphere), in relation to the plant species and the culture system (Mladenova et al., 2024).
The use of chemicals (e.g. pesticides) in horticultural ecosystems has utility for plant protection, according to the cropping system, but has also been associated with various negative effects on the environment and, sometimes, on plants and the quality of plant products (Ahmadi et al., 2024). Pesticides are inputs that indirectly influence the yield of horticultural crops, through plant protection (Wan et al., 2023).
The use of pesticides was analyzed in relation to the category of substances and the share used, in relation to the crops (vegetables), horticultural systems, farmer training, horticultural practices and technologies, protection and control aspects (weeds, diseases, pests) as well as from an economic perspective (Ngowi et al., 2007; Wan et al., 2023).
The relationship of horticultural plants with different chemical substances (contaminants) was analyzed in different plant organs (Li, 2022). The author reported different patterns of presence of the evaluated chemical elements, at the root level and at the leaf level. Reductions in plant root growth have been recorded in conditions of cadmium presence in the soil, in the rhizosphere area (Lux et al., 2011). Experimental supplementation of some mineral elements such as cobalt (Co), copper (Cu) and

zinc (Zn) led to their differentiated accumulation in the experimentally tested vegetable plants (Zakharova et al., 2023).
Among vegetables, onion is a plant of great importance worldwide (Bettoni et al., 2014; Cho et al., 2024). The onion root has a specific structure, with the role of fixing the plant, providing water and nutrients, and has a variable development in relation to the substrate and different influencing factors (Bailey et al., 2002). Morphological and genetic parameters at the root level in Allium plants were analyzed to evaluate the response of plants to different factors (Michalska-Kacymirow et al., 2014; Karasinski et al., 2017; Pokluda et al., 2023; Solouki et al., 2023; Maity et al., 2024). Self-defense mechanisms have been identified in onions in relation to biotic stress (Cho et al., 2024).
This study evaluated, in laboratory conditions, the response of *Allium cepa* plants to different chemical substances, based on morphological parameters of the root and leaf system.

MATERIALS AND METHODS

The research was organized and conducted under laboratory conditions, within the Genetics discipline, Universsity of Life Sciences “King Mihai I” from Timișoara, 2023. Onion bulbs, *Allium cepa* L., were placed in Petri dishes for vegetation, in distilled water. At the beginning of the experiment (T0, date February 21, 2023) a humid environment was provided with distilled water, in equal amounts. A control variant, with distilled water, was maintained (V1), for comparisons during the experiment. On February 24 (T1), observations were made on roots and coleoptiles. After the first observations, different chemicals were added, which differentiated the experimental variants; distilled water (V1), copper sulfate - $CuSO_4$ – 300 mg L^{-1} – V2), Faster insecticide (0.3 ml L^{-1} – V3, 0.4 ml L^{-1} – V4, 0.5 ml L^{-1} – V5) and Karate zeon insecticide (0.15 mg L^{-1} – V6; 0.20 mg L^{-1} – V7; 0.25 mg L^{-1} – V8). Morphological parameters (root length, RL; leaf number, LN; leaf length, LL) were determined. The determinations were made at five times (T2-T6), until March 12, 2023.
The values of the morphological parameters were recorded, and the experimental data set was analyzed by general statistical analysis, as well as by correlation, regression, multivariate analysis (Hammer et al., 2001; JASP, 2022), in order to quantify the response of the plants in the growth conditions generated by the chemical substances supplemented.

RESULTS AND DISCUSSIONS

Under the influence of the chemicals added to the growth vessels, morphological parameters of onion plants evolved differently. Starting from the moment T2, differentiations between variants were recorded for each monitored parameter (Table 1).

Table 1. Parameter values in *Allium cepa* in relation to experimental variants

Parameters	MU	V1	V2	V3	V4	V5	V6	V7	V8	±SE
BW	(g)	5.51	5.52	5.66	6.21	4.99	4.84	5.24	5.77	±0.16
BD	(mm)	22.61	22.55	22.54	22.91	22.05	21.39	21.94	22.50	±0.17
BH	(mm)	28.46	26.17	25.61	28.17	25.84	23.36	23.92	23.72	±0.69
RNT1	(no)	18.90	18.70	18.30	22.20	18.30	20.20	17.50	16.30	±0.63
RLT1	(cm)	1.41	1.30	1.08	1.48	1.38	1.51	1.28	1.05	±0.06
CLT1	(cm)	0.00	0.00	0.00	0.15	0.00	0.21	0.00	0.00	±0.03
RLT2	(cm)	4.34	1.35	1.10	1.49	1.47	2.08	1.81	1.64	±0.36
LLT2	(cm)	3.19	0.00	0.00	0.53	0.03	0.49	0.54	0.21	±0.38
LNT2	(no)	1.60	0.00	0.00	0.40	0.00	0.60	0.40	0.00	±0.19
RLT3	(cm)	7.96	1.48	1.28	2.08	5.29	4.50	2.64	4.80	±0.81
LLT3	(cm)	8.60	0.45	0.56	1.54	3.49	2.85	3.20	3.68	±0.91
LNT3	(no)	2.50	0.40	0.20	1.00	0.90	1.30	1.40	1.20	±0.25
RLT4	(cm)	7.96	1.48	1.28	2.08	5.29	4.50	2.64	4.80	±0.81
LLT4	(cm)	14.21	0.93	1.69	3.86	4.25	5.66	5.54	5.15	±1.43
LNT4	(no)	2.90	0.60	0.80	1.30	1.10	1.70	1.50	1.40	±0.25
RLT5	(cm)	16.68	1.66	5.18	4.64	8.30	12.40	11.62	9.28	±1.72
LLT5	(cm)	19.66	2.07	3.45	6.89	9.02	14.48	9.16	6.93	±2.03
LNT5	(no)	2.90	1.00	1.20	1.80	1.90	2.80	1.80	1.40	±0.24
RLT6	(cm)	18.49	1.70	7.68	7.16	9.97	14.09	13.81	10.80	±1.82
LLT6	(cm)	27.68	3.72	5.80	10.38	14.67	20.44	13.25	8.18	±2.81
LNT6	(no)	3.40	1.10	1.20	2.30	2.90	3.00	2.10	1.40	±0.31

Lower values of the parameters were recorded in variant V2, which indicates a higher level of stress in the case of this variant. The values of the parameters RL, LL, and LN, on the experimental variants, are shown in Table 1. The results reliability, as well as the presence of variance, were confirmed based on ANOVA Test (Table 2).

Different correlation levels were observed between *Allium* bulb parameters, as well as at the level of morphological parameters at time T1 (Figure 1). In contrast, positive correlations were recorded at the level of plant morphological parameters over the observation interval T2-T6 (Figure 1).

Table 2. Results from the ANOVA Test

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	8988.22	20	449.411	49.201	6.66E-55	1.6424
Within Groups	1342.73	147	9.134			
Total	10330.95	167				

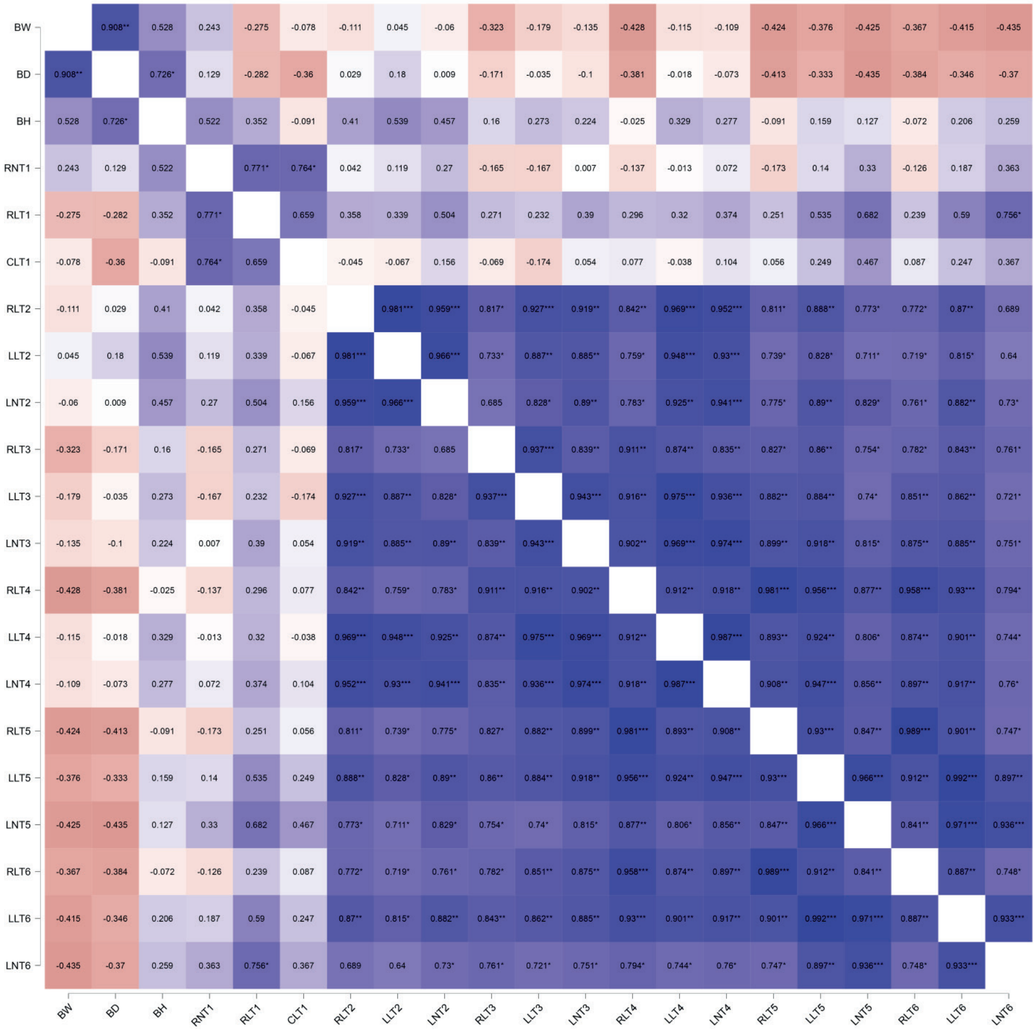

Figure 1. Correlations at the level of morphological parameters, *Allium cepa*

The control variant (V1) benefited from normal plant growth conditions, without chemical treatments. In this variant, high values of the parameters were recorded at the evaluation times, T1-T6. In the case of the RL parameter, the value $\overline{RL}$ =4.62 cm resulted, as the average of the experimental variants (Table 3). Compared to $\overline{RL}$, variant V1 presented positive differences, and variant V2 negative differences (p<0.01). In the case of the LL parameter, the value $\overline{LL}$=4.41 resulted, as the average of the experimental variants. Compared to $\overline{LL}$, the control variant (V1) presented positive differences (p<0.001), and the variants V2 and V3 negative differences (p<0.05), Table 3. In the case of the LN parameter, the result was $\overline{LN}$=0.99, as the average value of the experimental variants. Compared to $\overline{LN}$, the control variant (V1) presented positive differences (p<0.001), and the variants V2 and V3 presented negative differences (p<0.05), (Table 3).

Table 3. Significance of differences in analyzed parameters

Variant	Parameters					
	RL	Diff	LL	Diff	LN	Diff
V1	8.71	4.09^{**}	10.48	6.07^{***}	1.90	0.91^{***}
V2	1.30	-3.32^{oo}	1.02	-3.39^{o}	0.44	-0.55^{o}
V3	2.81	-1.81^{ns}	1.64	-2.77^{o}	0.49	-0.50^{o}
V4	2.89	-1.73^{ns}	3.34	-1.07^{ns}	0.97	-0.02^{ns}
V5	4.71	0.09^{ns}	4.49	0.08^{ns}	0.97	-0.02^{ns}
V6	6.22	1.60^{ns}	6.30	1.89^{ns}	1.34	0.35^{ns}
V7	5.41	0.79^{ns}	4.53	0.12^{ns}	1.03	0.04^{ns}
V8	4.91	0.29^{ns}	3.45	-0.96^{ns}	0.77	-0.22^{ns}

Under the influence of the applied chemicals, the variation of Allium plant parameters was described by polynomial models, with variable distribution in relation to the time factor (T, days).

The average growth rate ($\overline{AGR}$) was calculated for each parameter and experimental variant (Table 4). The $\overline{AGR}$ values for variants V2 to V8, under stress conditions generated by the chemicals used, were below the level of the control variant (V1).

The root system ensures the supply of water and nutrients to plants, and the reduction in the functioning of the root system influences the growth and development of plants (Macias-Leon and Leskovar, 2017; Atif et al., 2020; Solano et al., 2023).

Table 4. Average growth rate of parameters in *Allium cepa*

Experimental Variant	Average growth rate ($\overline{AGR}$)		
	$\overline{AGR_{RL}}$	$\overline{AGR_{LL}}$	$\overline{AGR_{LN}}$
V1	0.6603	0.3742	0.0679
V2	0.0607	0.0366	0.0158
V3	0.2743	0.0587	0.0173
V4	0.2557	0.1191	0.0347
V5	0.3561	0.1605	0.0347
V6	0.5032	0.2252	0.0480
V7	0.4932	0.1617	0.0367
V8	0.3857	0.1232	0.0276
SE	±0.065	±0.037	±0.006

The average growth rate of leaf length $\overline{AGR_{LL}}$ was described in relation to $\overline{AGR_{RL}}$, equation (1), with R^2 = 0.915, p = 0.0021, F = 27.053, and the average growth rate of leaf number $\overline{AGR_{LN}}$ was described in relation to $\overline{AGR_{RL}}$, equation (2), with R^2 = 0.855, p = 0.0079, F = 14.758.

$$\overline{AGR_{LL}} = 0.8683x^2 - 0.1044x + 0.04964 \quad (1)$$

$$\overline{AGR_{LN}} = 0.1272x^2 - 0.01103x + 0.01796 \quad (2)$$

where: x – average root growth rate ($\overline{AGR_{RL}}$)

$\overline{AGR_{LL}}$ parameter variation, based on $\overline{AGR_{LN}}$ values, was given by equation (3), with R^2 = 0.982, F = 139.85, p<0.001.

$$\overline{AGR_{LL}} = 29.51x^2 + 3.783x - 0.02047 \quad (3)$$

where: x – average rate of growth of the number of leaves ($\overline{AGR_{LN}}$)

Polynomial models and graphical models described the variation of the parameters RL, LL, LN in relation to time (T). The distribution of the control variant (V1) is presented compared to the variant V2, which recorded high level stress (Figures 2-4).

The variance was explained differentially, in relation to the principal components and morphological parameters, according to the values in Table 5. For example, the PCA diagram for the LL parameter was presented, in which the first two principal components (PC1, PC2) explained 97.617% of variance.

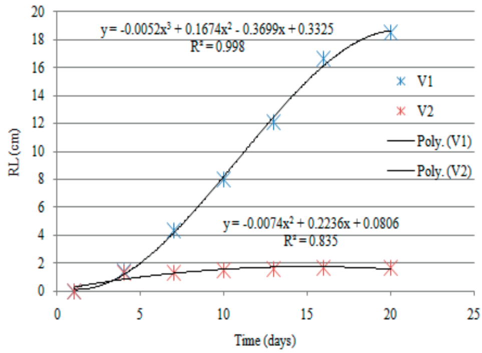

Figure 2. Root length (RL) parameter variation in relation to time

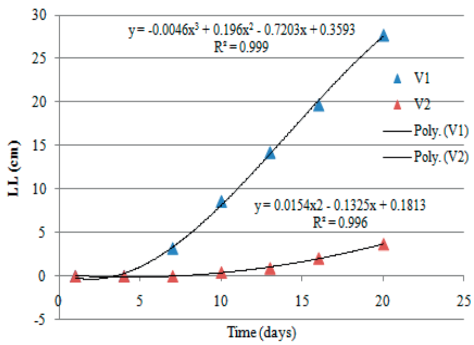

Figure 3. Leaf length (LL) parameter variation in relation to time

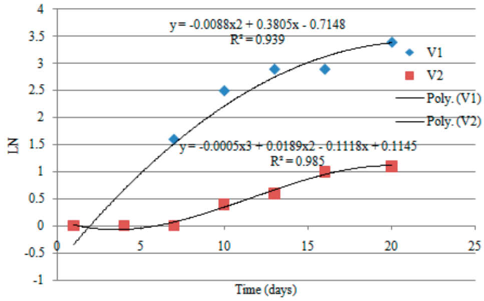

Figure 4. Leaves number (LN) parameter variation in relation to time

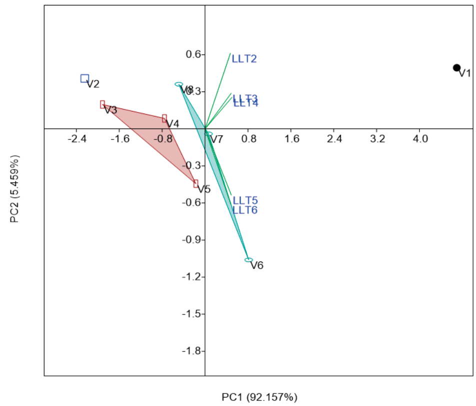

Figure 5. Principal component analysis (PCA) diagram in relation to the leaf length parameter, moments T2-T6

Table 5. Level of variance in relation to the principal components and morphological parameters

Principal Component (PC)	Parameters					
	Root length		Leaf length		Leaves number	
	Eigenvalue	% variance	Eigenvalue	% variance	Eigenvalue	% variance
1	4.371	87.426	4.608	92.157	4.397	87.932
2	0.391	7.823	0.273	5.460	0.423	8.466
3	0.230	4.601	0.107	2.140	0.118	2.363
4	0.007	0.150	0.011	0.213	0.050	1.003
5	6.87E-34	1.37E-32	0.002	0.030	0.012	0.236

The experimental variants were grouped into clusters (Figure 6), according to level of similarity, based on parameters values (RL, LL, LN), moments T1-T6 (Coph.corr. = 0.811). In stress-free conditions, plants from variant V1 recorded high values of the parameters RL, LL, LN in the test conditions. Thus, variant V1 was positioned independently of the other variants (Figure 6). The other variants were grouped into two sub-clusters. Variants V2 and V3, with similar levels of response to the stress generated by the chemicals, followed by variant V4, were grouped within a sub-cluster. The other variants, with intermediate values to the stress generated, were grouped into sub-cluster C2-B.

The chemicals used, in the tested concentrations, differentially influenced morphological parameters in onions, under laboratory conditions. The substances with the most pronounced repressive effect were $CuSO_4$ (300 mg L^{-1}) in variant V2, followed by Faster (0.3 ml L^{-1}) in variant V3.

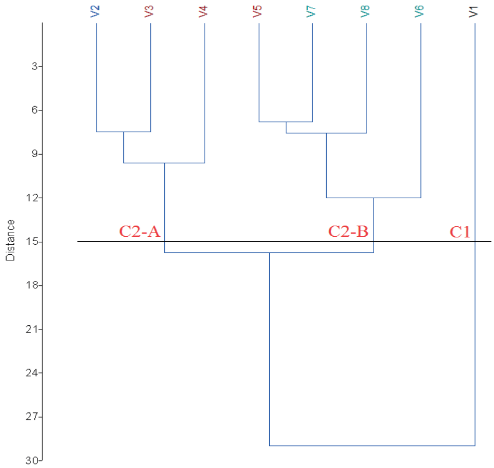

Figure 6. Cluster dendrogram based on Euclidean distances

The effect of Cu toxicity in onion roots was recorded depending on the concentration of Cu added under control, and the exposure time of the plants (Qin et al., 2015). Cu detection in onion leaves was studied in relation to different Cu concentrations provided in controlled germination medium (Jabbar et al., 2019). Copper supplementation to onions in different forms (nanoparticles, chelates, sulfate) showed that although $CuSO_4$ is the more accessible form, better effects were recorded in the case of Cu nanoparticles (Mottaleb et al., 2021). In a controlled environment, onion plant tolerance to low doses of Cu was recorded, and plant compromise with increasing concentration (Silva et al., 2022).

Within variant V4, the Faster product (0.4 ml L^{-1}), more pronounced repressive effects were recorded on the RL parameters, followed by LL, and the LN parameter recorded values at the average level of the experiment.

In the case of the Karate zeon product, variant V6 had values above the average of the experiment for all parameters, Variant V7 presented values above the average (RL parameter), and values at the average level (LL, LN parameters). In the case of variant V8 (0.25 mg L^{-1}) the Karate zeon product generated a stronger stress effect on the foliar parameters (LL, LN).

The RL and LL parameters showed higher sensitivity to $CuSO_4$ (300 mg L^{-1}) in variant V2, and to Faster (0.3 ml L^{-1}) in variant V3.

Under conditions of soils with high metal content (e.g. Cd), different levels of Cd in vegetable plants and mechanisms associated with Cd in plants were identified (Xiao et al., 2023). Onion and garlic showed lower Cd content in plants, as a result of mechanisms associated with Cd in the root (Xiao et al., 2023).

The average root growth rate (ΔRL) recorded the lowest values in variants V3 and V8, interval T1-T2. Variant V2 maintained low values of ΔRL throughout the observation interval.

In the case of the LL parameter, the average growth rate ΔLL recorded the lowest level in variants V2 and V3, the interval T1-T2. Variant V2 maintained low values of ΔLL throughout the observation interval.

In the case of the LN parameter, the average growth rate ΔLN recorded the lowest level in variants V2, V3 and V8 over the T1-T2 interval. Variant V2 maintained low values of ΔLN over the entire observation interval.

CONCLUSIONS

Differential effects were recorded in onions associated with chemical substances supplemented under controlled conditions.

A pronounced repressive effect was recorded in the case of $CuSO_4$ (300 mg L^{-1}, V2) on the RL parameter. The Faster product (0.3 ml L^{-1}, and 0.4 ml L^{-1}, V3, V4) generated more pronounced negative effects on RL, followed by LL and LN.

The Karate zeon product generated obvious repressive effects at a concentration of 0.25 mg L^{-1} (V8) on the LL and LN parameters.

The RL and LL parameters showed higher sensitivity to $CuSO_4$ (300 mg L^{-1}) in variant V2, and to the Faster product (0.3 ml L^{-1}) in variant V3.

The average growth rate of the studied parameters (ΔRL, ΔLL, ΔLN) was influenced by the substances added to the plant growth vessels. The lowest level was recorded in variant V2, under the influence of $CuSO_4$ throughout the study period. During the T1-T2 interval, variants V2, V3 and V8 presented reduced growth rate values.

The variation of the values of the parameters RL, LL, LN with respect to time was described by polynomial equations ($p<0.001$). Multivariate analysis explained the variance and association of variants based on similarity.

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