INCREASING DOSES OF GERMANIUM IN THE SOIL ALTER THE PRIMARY METABOLISM OF RADISH PLANTS

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ABSTRACT

Germanium (Ge) is a chemical element used in several industrial processes. According to the Survey of Rocks and Soils of the Geological Service of Brazil (SGB-CPRM) this element is found in a wide territorial range in the country. Thus, The objective of this study was to evaluate the primary metabolism of *Raphanus sativus* when grown with different doses of germanium, in relation to photosynthetic performance and growth for three crop cycles. The experiment consisted of growing radish in 7 treatments containing germanium in the soil at concentrations of 0; 0.5; 1; 1.5; 2; 2.5; 3 mg kg^{-1}. Analyses were performed over three complete cycles of the crop, from seedling production, transplanting and harvesting. The parameters analyzed in each cycle were: growth, chlorophyll concentration, chlorophyll a fluorescence, and stomatal conductance. The treatments with concentrations up to 1.5 mg kg^{-1}, in all cycles, presented a better performance. Indicating a possible toxicity for levels above this.

Keywords: *Raphanus sativus*, plant development, chlorophyll fluorescence *a.*

INTRODUCTION

Germanium (Ge) is not a rare element, but its geographical occurrence is dispersed in soils (WICHE et al., 2017) with basic characteristics, such as basalts, amphibolites and peatlands (SILVA et al., 2017). The content of Ge in the earth's crust is estimated to be 1.6 µg.g^{-1} (ROSENBERG, 2007), it was discovered in 1886, being widely used in the technology industry and until then it is one of the little studied elements mainly in the soil-plant system (TAO et al., 2021). This element can change its form of migration and availability depending on its physicochemical conditions, which makes its analysis even more difficult (SOBOLEV et al., 2020).

Ge is present in plants and animals. In the biogeochemical cycle, Ge presents properties and behavior very similar to silicon (Si), being considered cognate elements (ROSEMBERG, 2009). With respect to the soil-plant system, Ge behaves chemically like Si, being present mainly in aqueous solutions in the inorganic form of Ge, Ge (OH)4, similar to Si, Si (OH)4 (WICHE et al., 2018; SCHWABE et al., 2021).

In most soils, germanium is present in dizzying amounts, but certain types of soils may contain higher levels of germanium than others. The types of soils known to have relatively high levels of germanium are volcanic soils and soils rich in organic matter. The minerals richest in Ge are germanite and argyrodite, but as with soils, rocks, and these minerals, the concentration of Ge is low and widely dispersed in the earth's environment.

Thus, up to the present day, no commercial Ge mining site is known in the world. In Brazil, surveys and studies of Ge concentrations in Brazilian soils are extremely rare. Practically the only survey to determine this element in the country was conducted by the Geological Service of Brazil in the past decade (Silva et al. 2017). As expected, the work concluded that Brazilian soils present very low concentrations of Ge, reaching a maximum concentration of 1.7 $mg.kg^{-1}$.

Considering the few studies with the element in plant development (vegetables), the radish (*Raphanus sativus*), for being a crop with an extremely short cycle, is excellent for studies on plant development, easy to grow, coming from the Mediterranean region, it has a production cycle that lasts from 25 to 35 days and is a crop adaptable to different temperatures and can be grown in a range of 7.2 to 32.2 °C, 29.4 °C being considered ideal for planting (MINAMI & NETTO, 1997).

The objective of this study was to evaluate the primary metabolism of *Raphanus sativus* when grown with different doses of germanium, in relation to photosynthetic performance and growth in three crop cycles.

MATERIAL AND METHODS

The experiment was developed on the Gragoatá campus of the Universidade Federal Fluminense. The region has an Aw climate, according to the Köppen classification, that is, a tropical climate with dry winters and rainy summers, with a mean annual temperature of 23ºC and precipitation of 1200 mm. The location has latitude 22° 54' 00'' S, longitude 43° 08' 00'' W and an altitude of 8 meters.

The soil used for the experiment was from the municipality of Maracaju/MS, characterized as Latossolo Vermelho medium texture, according to the Brazilian Soil Classification System (EMBRAPA, 2013). The plants were grown in plastic pots with a capacity of 4 dm^3, where it was decided not to have a drainage system in the pots to avoid loss of the chemical element of interest. A weight of 3.86 kg of soil per pot was adopted, and the weight of each pot at field capacity was 5 kg.

The radish seeds (*Raphanus sativus*) used were of commercial origin. The experiment was conducted in a greenhouse with 70% shade. The seedlings were prepared in polyethylene trays with 200 cells, and transplanted into pots eight days after sowing. Initially, three seedlings were transplanted per pot, arranged in triangular position and after ten days one of the plants was thinned, as recommended by Filgueira (2009), who recommends that when the plants are 5 cm high, thinning should be carried out, this choice being made by analyzing the one that had less vigor, leaving, in this case, two plants per pot.

For this, the experiment consisted of an entirely randomized design, with seven treatments of germanium doses: 0; 0.5; 1.0; 1.5; 2.0; 2.5 and 3.0 $mg.kg^{-1}$ and three repetitions. In each treatment, it was considered 2 experimental units (plants) per vase, totaling 6 repetitions per treatment.

Fertigation was done at transplanting, adding to each pot, 200 ml of the soluble fertilizer "plant pro" of composition NPK 15-30-15 with a dosage of 35 g/100 L, in all pots equally. Besides the addition of NPK macronutrients, this fertilizer also provides several micronutrients essential for plant growth: 15% Total nitrogen (N); 30% available phosphoric acid (P2O5); 15% Soluble potash (K2O); 0. 02% boron (B); 0.05% chelated copper (Cu); 0.10 chelated iron (Fe); 0.05% chelated manganese (Mn); 0.0005% molybdenum (Mo); 0.05% chelated zinc (Zn) and 1% EDTA - ethylene diamine tetraacetate (chelating agent).

The replacement of water via irrigation occurred on alternate days by adding 150 ml per vase. Once a week, the pots were weighed using the gravimetric method to establish the amount of water needed to replace the water to field capacity and, by calculating evapotranspiration, water was replaced according to the value determined for each vase.

Three crop cycles were performed in order to evaluate the primary metabolism of radish plants under the influence of different doses of Ge in the soil throughout the cycles, as described in Table 1, and all plant material collection and analyses were performed on the last date of each cycle.

The temperature data (ºC) over the three cycles were collected from an indoor weather station, model IRRIPLUS®, using daily data of maximum, minimum and average temperature and treated in EXCEL software.

The growth parameters (height, diameter of the neck, leaf area, number of leaves, fresh mass and dry mass) were measured weekly from 7 DAT (days after transplanting) for four repetitions of each treatment: i. Height: Height was measured using a tape measure, from the neck to the length of the largest leaf, and the value was expressed in (cm); ii. Diameter of the neck: The diameter was checked using a pachymeter at the point of the neck closest to the root, with four repetitions per analysis and expressed in (mm); iii. Leaf area: The leaf area was verified using the formula= {A= a+b.L. W} (SALERNO et al., 2005), where a and b were coefficients found in order to obtain a linear equation for the leaf area, L the length of the plant and W the width, and the value was expressed in (cm^2 $plant^{-1}$); iv. Number of leaves: The number of leaves was obtained from 4 repetitions, by direct counting of the fully open leaves and expressed in units; v. Fresh and dry mass: At the end of the experiment,

Table 1. Radish growing periods throughout the year 2022.

Season	1st Cycle	2nd Cycle	3rd Cycle
Summer	March 1st to 21st	-	-
Fall	-	May 5th to 27th	-
Winter	-	-	June 6th to 26th

the masses were verified using digital scales at the time of collection. The drying of the aerial part and root was performed separately, in a forced ventilation oven at a temperature of 65±2 ºC during the period of two days to reach the constant weight, and the two plants of the treatment were weighed together, the results were expressed in (kg).

Analysis of chlorophyll fluorescence a, was performed weekly in the three cycles on the same leaves that were selected for the determination of leaf area. These measurements were performed using a portable fluorometer (Handy PEA model, Hansatech Instruments, King's Lynn, Norfolk, UK) on 15 leaves, in the early morning, starting at seven o'clock, and these leaves were adapted to the dark with the use of clips during a period of thirty minutes for the oxidation of the photosynthetic system of electron transport. During the use of the apparatus for the measurements, the clips are opened and the leaves are exposed to a beam of light. Each of these repetitions had 1s analyses of the fluorescence emission in order to verify the fast fluorescence results based on the JIP test developed by Strasser and Strasser (1995) and Tsimilli-Michael & Strasser (2008).

The chlorophyll concentration was also verified by means of SPAD reading, where they were checked weekly using a Minolta SPAD-502 portable chlorophyllometer and its measurements were made on three different leaves per plant, considering only the lower

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two thirds of the leaf. The average SPAD index of each plant was obtained by averaging the three values and given by the equipment itself in the unit µg cm^{-2}.

The analysis of stomatal conductance was performed at the end, measured in the most expanded leaves, avoiding the central vein, in four repetitions of each treatment. Using the SC-1 Leaf Porometer (Decagon Devices), obtaining values expressed in mmol m^{-2} s^{-1}.

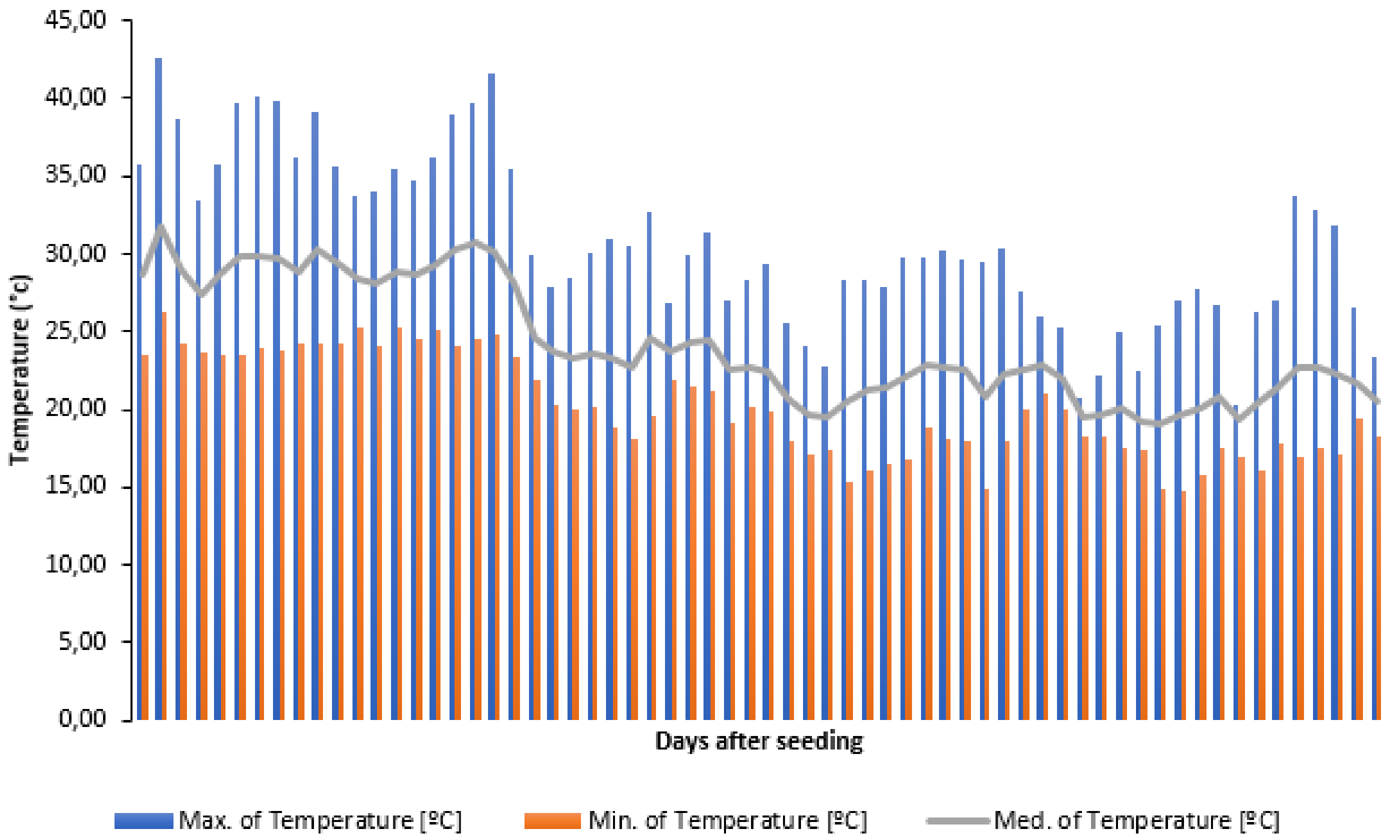
The data were tabulated in Excel spreadsheet and then analyzed by the Shapiro-Willk normality test, aiming to verify if they followed the preconditions for applying parametric variance tests. As the data presented normal distributions, they were submitted to analysis of variance (ANOVA). After the application of this test, when significant, the means were compared by the Tukey test at 5% probability level. All statistical analyses were performed using the SISVAR® software.

RESULTS AND DISCUSSION

The temperature data for the three cycles of the experimental period (Figure 1) are expressed as daily maximum, minimum and average temperature. The temperature data show the clear transition between the summer season and the beginning of winter during the experimental period, maintaining an average for the three cycles between 31.54ºC and 19.01ºC. Being this factor of utmost importance for the cultivation of vegetables, in this study it was a parameter of low impact due to the wide temperature range that the radish supports during its cultivation.

The three cycles showed no statistical difference for height in any of the treatments (Figure 2 A, B and C). Regarding collar diameter (Figure 2 D, E and F) it is observed that there were statistical differences between

Figure 1.
Daily maximum, minimum and average air temperature data for the city of Niterói between March 1, 2022 and June 25, 2022.



treatments in cycles 2 and 3, where doses above 1 mg kg^{-1} of Ge showed a lower performance in cycle 2, and doses above 0.5 mg kg^{-1} of Ge in cycle 3 had the same performance.

The diameter of the neck of the stem showed statistical differences between the treatments in cycles 2 and 3, and decreased in all treatments over the three cycles, becoming very evident in cycle 3 (Figure 2 F) where the values did not reach 7 mm in any of the treatments, being the result much lower when compared to the others.

Regarding the number of leaves (Figure 3 A, B and C), there were no statistical differences among the treatments in cycles 1 and 2. The number of leaves throughout the cycles had a small reduction in most treatments, being more noticeable when comparing cycles 1 and 3.

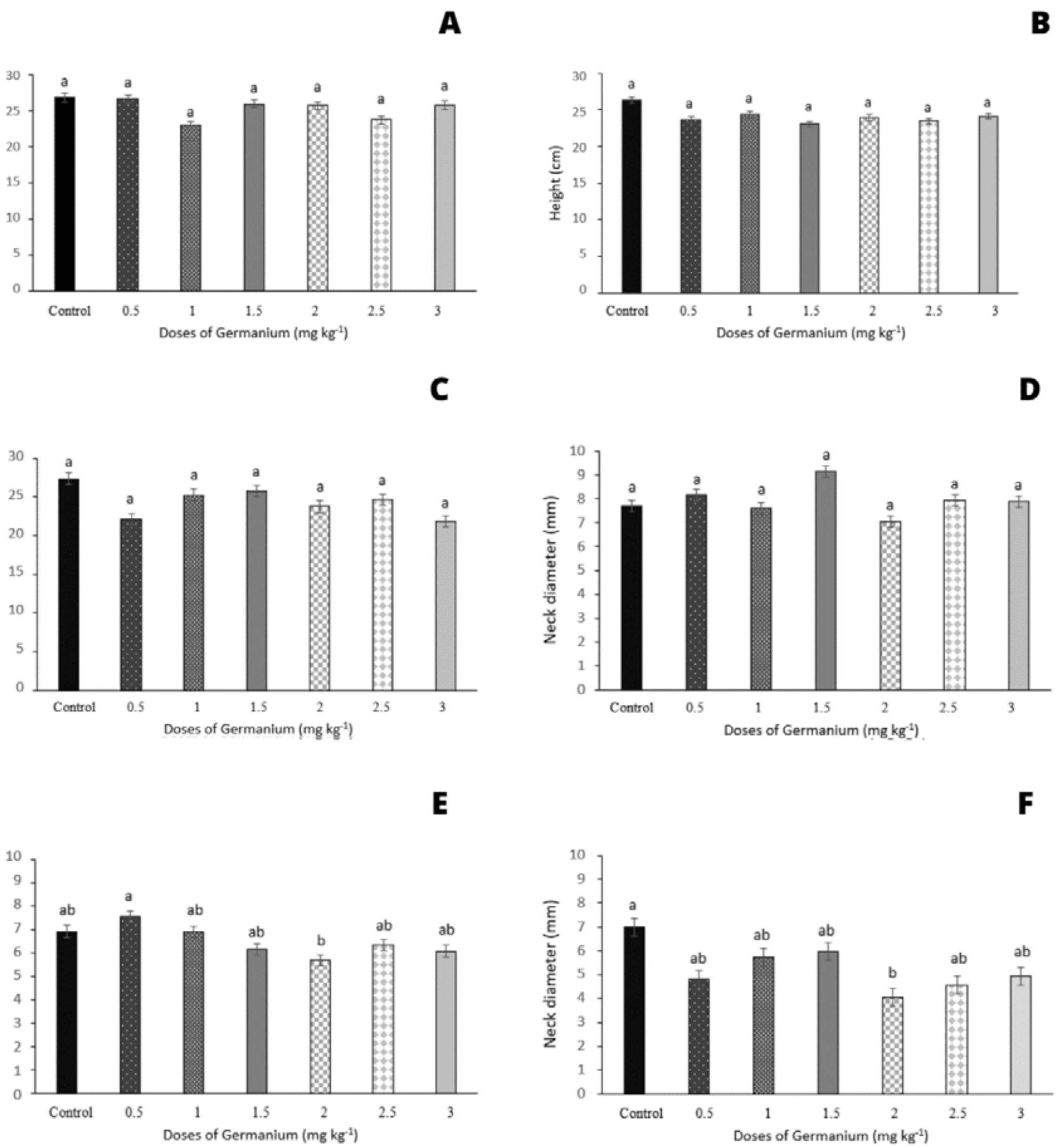


Figure 2. Height of the aerial part and diameter of the stem neck of radish (*Raphanus sativus*) cultivated in different doses of germanium, during three cycles. Aboveground height in cycle 1 (A); cycle 2 (B); cycle 3 (C); and stem neck diameter in cycle 1 (D); cycle 2 (E); cycle 3 (F). Equal letters do not statistically differ by the Tukey test at 5%.

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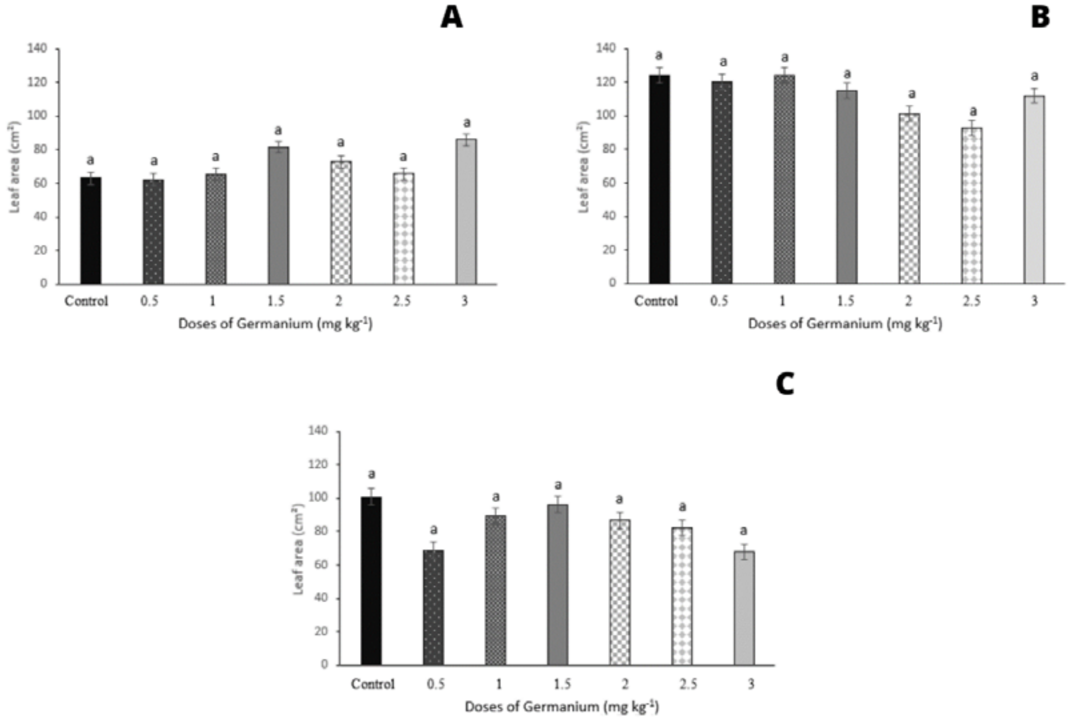


Figure 3.
Number of leaves of radish (*Raphanus sativus*) grown on different germanium doses during three cycles. Number of leaves in cycle 1 (A); cycle 2 (B); cycle 3 (C). Equal letters do not differ statistically from each other by Tukey's test at 5%.

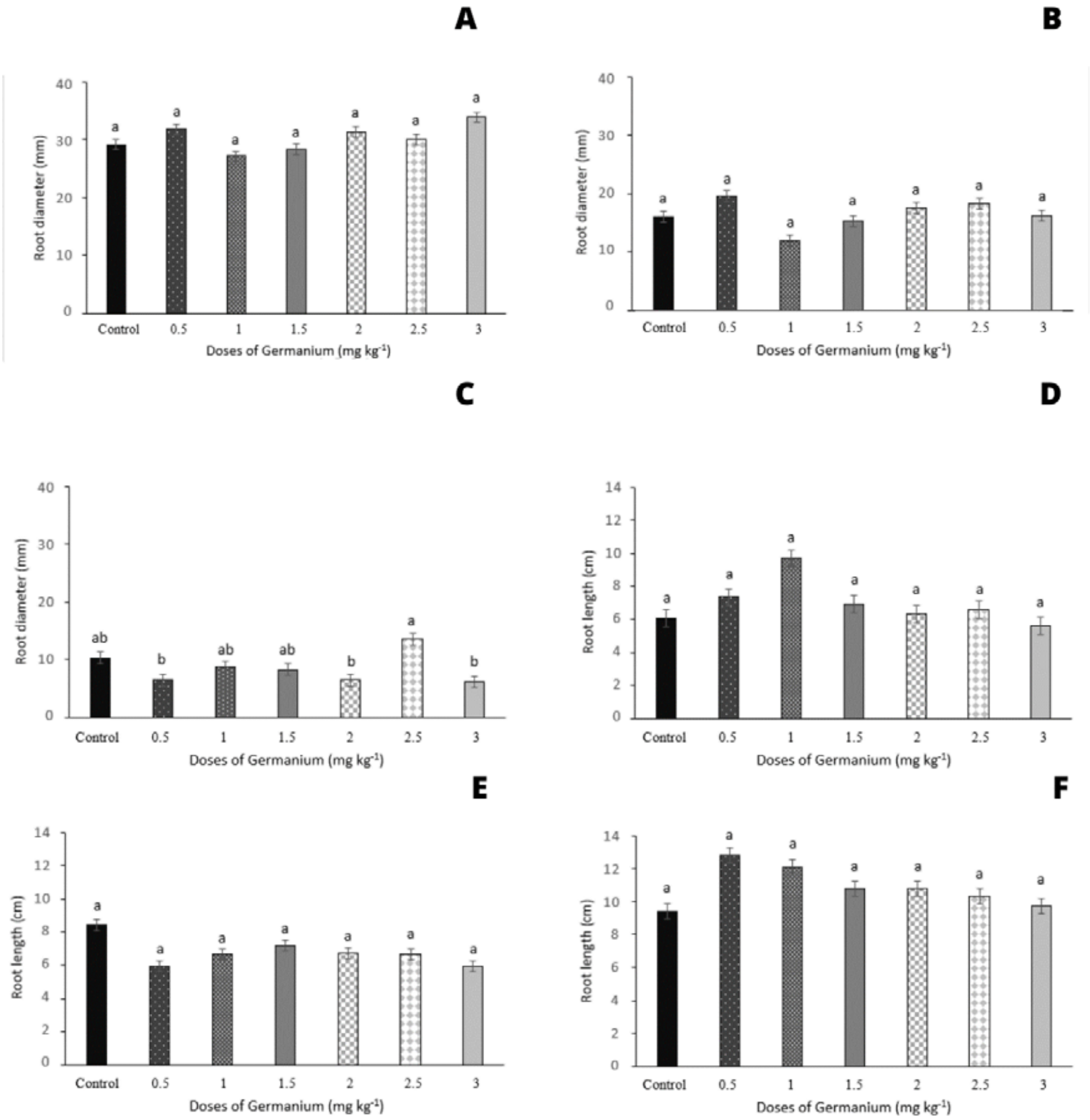
It was possible to see that as the number of leaves on the plant was smaller, its stem neck also decreased, and there is a study by Sun et al. (2019) that shows this correlation of the influence of the aerial part on the development of the stem neck, so as to balance the upper part with its base.

At the end of each cycle, root diameters (Figure 4 A, B and C) in all treatments showed a considerable reduction from one cycle to another, with no statistical differences between the treatments of cycles 1 and 2. In cycle 3, the treatments presented statistical differences among themselves, and this cycle presented a lower performance when compared to the previous ones.

While it can be observed in the previous figure the reduction of the radish root diameter that is its commercial product, its length (Figure 4 D, E and F) presented an inversely proportional performance and there were no statistical differences among treatments in any of the cycles. Moreover, in all three cycles, the treatments above the dose of 1.5 mg kg^{-1} were the ones that presented the lowest performance for both measurements. The leaf area (Figure 5 A, B and C) did not show statistical differences among the treatments, in any of the three cycles. The first cycle presented the smallest areas in all treatments, while the second cycle presented a better development when compared to the first and third cycles. In all cycles it is noticeable an alteration in the development of the leaves starting at the dose of 1.5 mg kg^{-1}.

The absence of germanium (Ge) replacement in cycles 2 and 3, may have been a factor that influenced the plant development compared to the first cycle. However, it is possible to verify that there was an alteration in the parameters of height, neck diameter, number of leaves, root length and leaf area in each of the cycles from the dose of 1.5 mg kg^{-1}, indicating a possible toxicity by the addition of the element Ge, studies performed by Cheong

Figure 4. Root diameter and root length of radish (*Raphanus sativus*) grown on different germanium doses for three cycles. Root diameter in cycle 1 (A); cycle 2 (B); cycle 3 (C); Root length in cycle 1 (D); cycle 2 (E); cycle 3 (F). Equal letters do not statistically differ by Tukey test at 5%.



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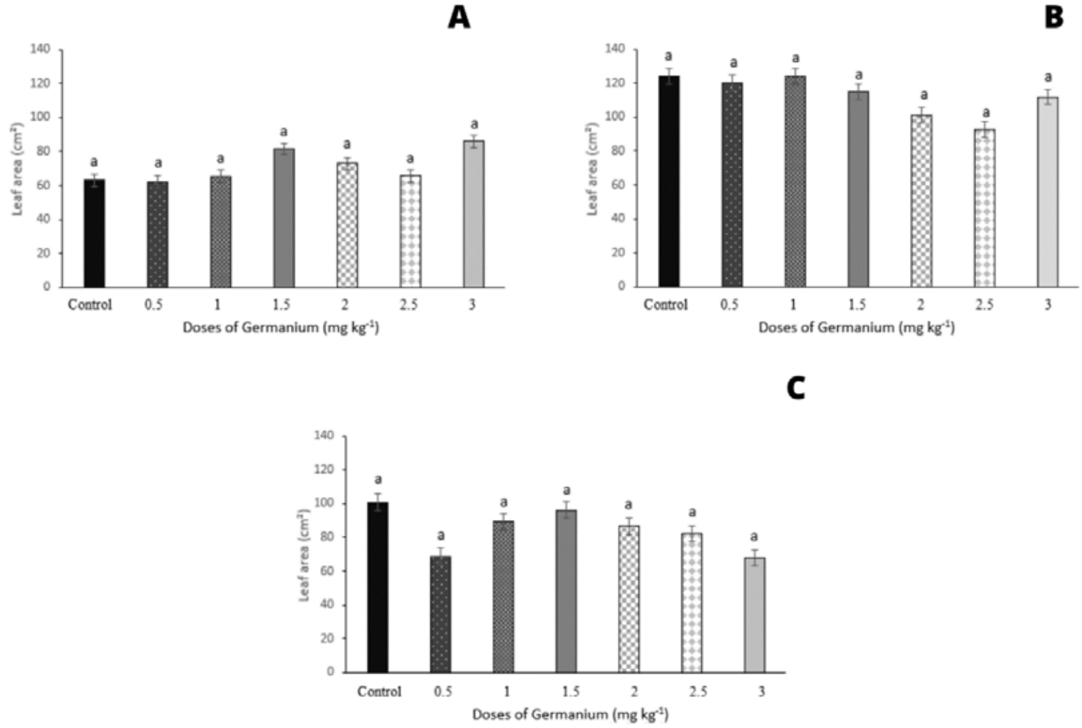

Figure 5.
Leaf area (cm²) of radish (*Raphanus sativus*) grown on different germanium doses during three cycles. Leaf area of cycle 1 (A); cycle 2 (B); cycle 3 (C). Equal letters do not differ statistically from each other by Tukey's test at 5%.

et al. (2009) showed negative alterations in the development of hydroponic vegetables in doses above 2.5 mg L^{-1} of Ge.

When the chlorophyll concentration data were analyzed (Figure 6 A, B and C), only cycle 2 showed statistical differences between its treatments, cycle 1 obtained higher chlorophyll rates in all its treatments and apparently the Ge dosages did not interfere in the production of chlorophyll in the plant. Stomatal conductance, which represents the gas exchange performed by the plant (Figure 6 D, E and F), did not show statistical differences between the treatments of cycles 1 and 2. Cycle 3 was the only cycle that showed statistical differences between treatments for stomatal conductance, where all treatments showed a lower performance when compared to the control.

The concentration of chlorophyll in the plants showed a reduction in all treatments in cycles 2 and 3, which can be correlated with the lower gas exchange that influences a lower production of photosynthesis, making the production of chlorophyll more precarious. A study conducted with the bell pepper crop showed that in fact the reduction of gas exchange, as well as the low availability of nutrients can directly impact the production of chlorophyll (SILVA et al., 2020).

The aboveground plant biomass (Table 2) showed statistical differences between treatments for dry mass in cycles 1 and 3. The fresh mass in cycle 1 presented statistical differences among treatments, which was even more accentuated in its dry mass. In the three cycles, it was possible to see that more than 90% of the radish leaf consists of water.

For a better comparison, the dry masses (Table 3) of each treatment between the three cycles were verified. Cycles 2 and 3 presented the same statistical differences in almost all treatments, except for the dose of 0.5 mg kg^{-1} where the three cycles differed significantly.

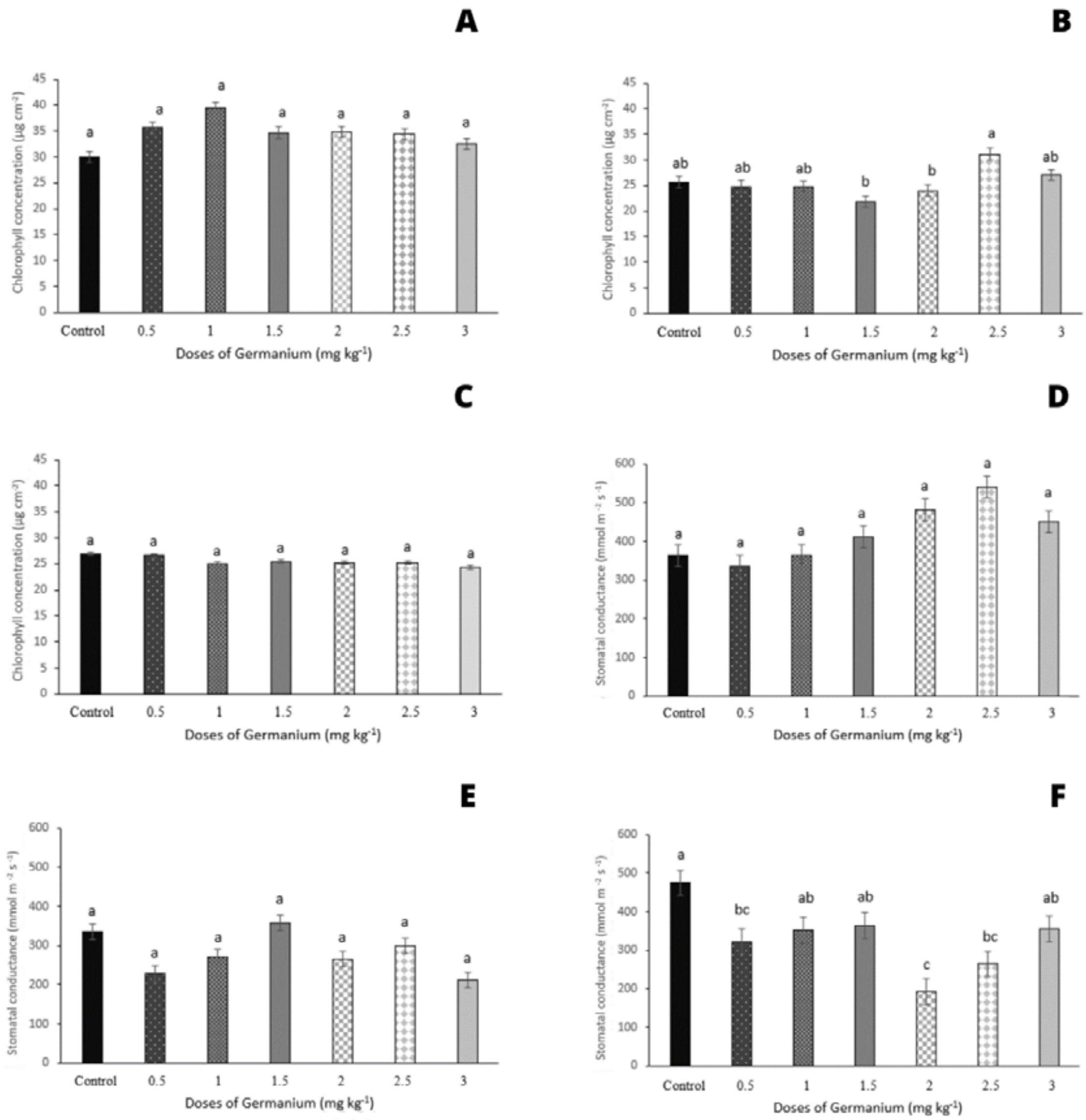

Figure 6.
Chlorophyll concentration (SPAD) and stomatal conductance of radish (*Raphanus sativus*) grown on different germanium doses during three cycles. Chlorophyll concentration in cycle 1 (A); cycle 2 (B); cycle 3 (C); and stomatal conductance in cycle 1 (D); cycle 2 (E); cycle 3 (F). Equal letters are not statistically different from each other by Tukey's test at 5%.

Table 2.
Aboveground biomass.

		CYCLE 1		CYCLE 2		CYCLE 3	
	Dose of Ge (mg kg^{-1})	Fresh Mass (g)	Dry Mass (g)	Fresh Mass (g)	Dry Mass (g)	Fresh Mass (g)	Dry Mass (g)
T1	Controle	40 ab	3.13 ab	35 a	2.20 a	30 a	1.78 a
T2	0.5	40 ab	3.14 ab	33 a	2.13 a	20 a	1.86 ab
T3	1	36 ab	2.93 ab	29 a	1.99 a	26 a	1.32 ab
T4	1.5	43 ab	3.69 a	27 a	1.81 a	30 a	1.59 ab
T5	2	46 a	3.44 a	23 a	1.63 a	23 a	1.26 ab
T6	2.5	26 b	2.10 b	28 a	1.95 a	23 a	1.26 b
T7	3	40 ab	3.13 ab	28 a	1.91 a	20 a	1.03 b

** Means followed by the same lower case letter in the columns do not differ by Tukey's test at 5% probability*

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Table 3. Aboveground dry biomass.

Dry Mass (g) Aerial Part				
	Dose of Ge (mg kg^{-1})	CYCLE 1	CYCLE 2	CYCLE 3
T1	Controle	3.13 A	2.20 B	1.78 B
T2	0.5	3.14 A	2.13 B	1.86 C
T3	1	2.93 A	1.99 B	1.32 B
T4	1.5	3.69 A	1.81 B	1.59 B
T5	2	3.44 A	1.63 B	1.26 B
T6	2.5	2.10 A	1.95 AB	1.26 B
T7	3	3.13 A	1.91 B	1.03 B

**Means followed by the same capital letter in the rows do not differ by Tukey's test at 5% probability*

Table 4. Root Biomass

		CYCLE 1		CYCLE 2		CYCLE 3	
	Dose of Ge (mg kg^{-1})	Fresh Mass (g)	Dry Mass (g)	Fresh Mass (g)	Dry Mass (g)	Fresh Mass (g)	Dry Mass (g)
T1	Control	26 a	1.58 a	10 a	0.54 a	3 a	0.19 a
T2	0.5	40 a	2.15 a	11 a	0.50 a	2 a	0.09 a
T3	1	36 a	1.85 a	40 a	0.25 a	2 a	0.10 a
T4	1.5	26 a	1.78 a	8 a	0.36 a	3 a	0.21 a
T5	2	30 a	1.91 a	6 a	0.28 a	3 a	0.18 a
T6	2.5	26 a	1.39 a	10 a	0.56 a	3 a	0.22 a
T7	3	33 a	1.88 a	10 a	0.33 a	6 a	0.20 a

**Means followed by the same capital letter in the rows do not differ by Tukey's test at 5% probability*

Table 5. Root Dry Biomass

Dry mass (g) Root				
	Dose of Ge (mg kg^{-1})	CYCLE 1	CYCLE 2	CYCLE 3
T1	Control	1.58 A	0.54 B	0.19 B
T2	0.5	2.15 A	0.50 B	0.09 B
T3	1	1.85 A	0.25 B	0.10 B
T4	1.5	1.78 A	0.36 B	0.21 B
T5	2	1.91 A	0.28 B	0.18 B
T6	2.5	1.39 A	0.56 B	0.22 B
T7	3	1.88 A	0.33 B	0.20 B

**Means followed by the same capital letter in the rows do not differ by Tukey's test at 5% probability*

The root biomass (Table 4), on the other hand, did not show statistical differences for the treatments in any of the cycles, and presented the same proportion of water as the aerial part, with a great loss of weight after drying.

When comparing the treatments among themselves for root dry mass (Table 5), the same pattern as the aboveground was observed. Cycle 1 presented the greatest mass for all treatments, differing from cycles 2 and 3, where despite there being a decrease in weight from one cycle to another, both did not differ statistically.

According to the data of growth parameters and biomass production presented, cycle 1 was the one that presented the best performance. The length of the root was the only factor in which cycle 1 obtained less growth compared to the other cycles, and this is considered positive because the lesser development of the length was inversely proportional to the diameter of the root, which is the commercial factor in question, thus cycle 1 presented the best diameters and greater biomass, while cycles two and three did not develop so well, indicating a sign of stress. Manzoor et al. (2021) point out that these deformations that occur in radish culture are an expressive sign of stress that can be linked to several factors.

Regarding the data from the chlorophyll-a fluorescence analysis (Figure 7 A, B and C), cycles 2 and 3 presented the greatest changes, showing great variations in the parameters F_v/F_m, F_v/F_0, ABS/RC, DI_0/RC, φE_0, φP_0, PI_{abs} and PI_{total}.

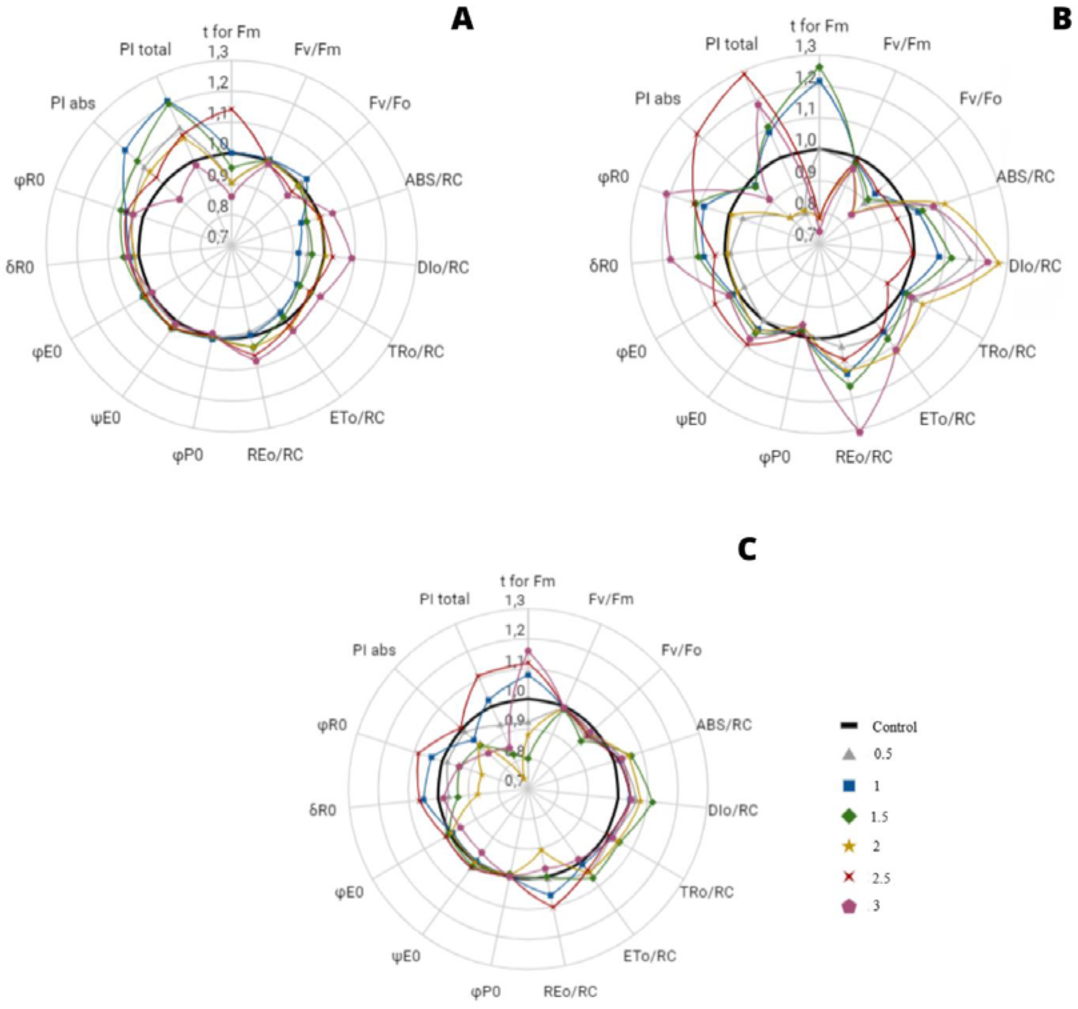

Figure 7.
Chlorophyll a transient fluorescence parameters, relative to the respective control, of radish (*Raphanus sativus*) grown on different germanium doses of 0; 0.5; 1.0; 1.5; 2.0; 2.5; 3.0 mg kg^{-1}.

The parameter (t for Fm) of time to reach maximum fluorescence showed values below and above the control in all cycles, and in cycle one, only treatment 3 responded within the expected time, treatments 2 (0.5 mg kg^{-1} Ge), 4 (2 mg kg^{-1} Ge), 5 (2.5 mg kg^{-1} Ge), and 7 (3 mg kg^{-1} Ge) showed a result below the control, showing a longer time to reach Fm. In cycle two, treatments 5 (2 mg kg^{-1} Ge), 6 (2.5 mg kg^{-1} Ge), and 7 (3 mg kg^{-1} Ge) took longer to reach Fm, and in cycle three, treatments 2 (0.5 mg kg^{-1} Ge), 4 (1.5 mg kg^{-1} Ge), and 5 (2 mg kg^{-1} Ge) were also below the control.

The levels of reason Fv/Fm, that indicate maximum photosynthetic efficiency of the FSII (photosystem II), was within control for all treatments in all three cycles. Cycle 1 showed values of F_v/F_0 above control for treatment 3 (1 mg kg^{-1} Ge) and values below for treatments 6 (2.5 mg kg^{-1} Ge) and 7 (3 mg kg^{-1} Ge). Cycles 2 and 3, on the other hand, showed values below the control for all treatments.

For the absorption flow per reaction center (ABS/RC) parameters and the energy dissipation in the form of heat (DI_0/RC), cycle 1 showed elevation in both, for treatment 7 (3 mg kg^{-1} Ge), while cycle 2 had an increase in treatments 2 (0,5 mg kg^{-1} Ge), 3 (1 mg kg^{-1} Ge), 4(1.5 mg kg^{-1} Ge), 5 (2 mg kg^{-1} Ge) and 7(3 mg kg^{-1} Ge). Cycle 3 showed an increase in these values in all cycles.

The parameter Et_0/RC, indicates the electron transport flux beyond QA- per active reaction center only the third cycle showed treatments equal to the control, these being 3 (1 mg kg^{-1} Ge) and 7 (3 mg kg^{-1} Ge).

RE_0/RC, is the specific electron flux with the capacity to reduce the final electron acceptors in the electron acceptor portion of the ISF per active reaction center. Cycle 1 presented the treatments 2 (0.5 mg kg^{-1} Ge) and 3 (1 mg kg^{-1} Ge) within the control. In Cycle 2 all treatments presented values above the control, while in Cycle 3, treatments 2 (0.5 mg kg^{-1} Ge) and 4 (1.5 mg kg^{-1} Ge) within the control.

Regarding chlorophyll fluorescence a alterations were observed in photosystem II that interfere with the adequate production of the photosynthesis process. The transport of electrons in cycles 1 and 2 was insufficient in all treatments, while the electron flow was efficiently attended until the third dose in cycle 1. Thus the other parameter that is the photosynthetic performance index (PI) depends on the reaction center and the electron transport (OUKARROUM et al., 2007) that can be identified in the analysis of the parameter RE_0/RC, being efficient in highlighting possible stresses by analyzing the absorption system and the use of energy in transport.

The Performance Index on an Absorption basis (PI_{ABS}) and the Total Performance Index (PI_{TOTAL}) when analyzed over the three cycles shows that the treatment 7 (3 mg kg^{-1} Ge) showed values below the control.

The Performance Index on an Absorption basis (PI_{ABS}) and the Total Performance Index (PI_{TOTAL}), show how this electron transport system reaches the ISP. In all three cycles the treatment 7 (3 mg kg^{-1} Ge) presented values below the control, which may be justified by the higher concentration of germanium that can cause toxicity to the plant, which may have influenced the lower production of biomass in treatments higher than 1.5 mg kg^{-1} because PI_{TOTAL} is a more sensitive indicator of stress in the plant as reported in a study developed by OLIVEIRA et al. (2018).

CONCLUSIONS

The use of different doses of germanium for studies with radish shows to be efficient, until the treatment of 1.5 mg kg^{-1}, being that, above this dose the photochemical activity is influenced negatively, indicating, perhaps, a possible toxicity by the excess of this element in the solution of the soil. Although not yet demonstrated by the other data, the analysis of chlorophyll fluorescence shows a noticeable alteration in the electron transport chain of photosynthesis. This alteration is one of the main indications of stress in plants.

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