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Initial development of peanut seedlings grown in soil with herbicide residue

Desenvolvimento inicial de plântulas de amendoim cultivadas em solo com resíduo com herbicida

Desarrollo inicial de plántulas de maní cultivadas en suelo con residuos de herbicidas

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Abstract
Peanut has been used as an alternative in crop rotation in large areas of sugarcane. Plants may be subject to residual herbicides in these commercial areas as 2,4D and Picloram are applied for weed control. The objective of this research was to know the initial development of peanut seedlings grown in soil with herbicide residue. The experiment was carried out in August 2022, at Faculdades Integradas Stella Maris (FISMA), located in the Municipality of Andradina, State of São Paulo. The design was completely randomized (DIC), with five doses of the commercial product Dontor® (Picloram Acid and 2,4D Acid) as follows: absence; 1.0; 2.0; 4.0 and 8.0 L ha-1 and with four replications, totaling 20 plots or pots. The maximum point in the peanut response was at the dose of 5.0 liters per hectare of the herbicide 2,4D + Picloram. A dose greater than 5.00 liters per hectare of the herbicide 2,4D + Picloram compromises the development of peanuts. Leaf area and stomatal density responded in a negative linear fashion to herbicide doses.
Keywords: *Arachis hypogaea*; 2.4D; Picloram; Phytotoxicity; Residual.

Resumo
O amendoim vem sendo utilizado como uma alternativa na rotação de cultura em grandes áreas de cana-de-açúcar. As plantas podem estar sujeitas a residual de herbicidas nessas áreas comerciais, pois o 2,4D e Picloram são aplicados para o controle de plantas daninhas. O objetivo dessa pesquisa foi de conhecer o desenvolvimento inicial de plântulas de amendoim cultivadas em solo com resíduo com herbicida. O experimento foi realizado em agosto de 2022, nas Faculdades Integradas Stella Maris (FISMA), localizada no Município de Andradina, Estado de São Paulo. O delineamento foi inteiramente casualizado (DIC), com cinco doses do produto comercial Dontor® (Ácido de Picloram e Ácido de 2,4D) sendo elas: ausência; 1,0; 2,0; 4,0 e 8,0 L ha^{-1} e com quatro repetições, totalizando 20 parcelas ou vasos. O ponto de máxima na resposta do amendoim foi na dose de 5,0 litros por hectare do herbicida 2,4D + Picloram. Dose superior a 5,00 litros por hectare do herbicida 2,4D + Picloram compromete o desenvolvimento do amendoim. A área foliar e a densidade estomática responderam de maneira linear negativa nas doses de herbicida.
Palavras-chave: *Arachis hypogaea*; 2,4D; Picloram; Fitotoxidade; Residual.

Resumen
El maní se ha utilizado como alternativa en la rotación de cultivos en grandes extensiones de caña de azúcar. Las plantas pueden estar sujetas a herbicidas residuales en estas áreas comerciales ya que se aplican 2,4D y Picloram para el control de malezas. El objetivo de esta investigación fue conocer el desarrollo inicial de plántulas de maní cultivadas en suelo con residuo de herbicida. El experimento se llevó a cabo en agosto de 2022, en las Faculdades Integradas Stella Maris (FISMA), ubicadas en el Municipio de Andradina, Estado de São Paulo. El diseño fue completamente al azar (DIC), con cinco dosis del producto comercial Dontor® (Ácido Picloram y Ácido 2,4D) así: ausencia; 1,0; 2.0; 4.0 y 8.0 L ha-1 y con cuatro repeticiones, totalizando 20 parcelas o macetas. El punto máximo en

la respuesta del maní fue a la dosis de 5,0 litros por hectárea del herbicida 2,4D + Picloram. Una dosis superior a 5,00 litros por hectárea del herbicida 2,4D + Picloram compromete el desarrollo del maní. El área foliar y la densidad estomática respondieron de forma lineal negativa a las dosis de herbicida.
Palabras clave: *Arachis hypogaea*; 2.4D; Picloram; Fitotoxicidad; Residual.

1. Introduction

With each passing day, sugarcane cultivation has been increasing its occupation of production area, using a large extension of land, which brings as a consequence the agronomic effects generated by monoculture. An effective solution to alleviate this problem is crop rotation with leguminous plants (Zang *et al.*, 2022).

Peanut is one of the main leguminous crops produced in several Brazilian regions, it has a fast cycle, in some cases it can have the production of up to two cycles in the same year, divided into rainy season and dry season (Lisboa *et al*., 2021).

One of the crops widely used as rotation in the sugarcane renewal cycle is the planting of peanuts, a plant that helps maintain and improve the physical, chemical and biological characteristics of the soil, even in depth, increasing the presence of microorganisms in the soil, which The cultivation of peanuts in the sugarcane field renovation period provides the producer with an economic value due to the plant improving soil aspects and also bringing financial return, production and savings with soil maintenance, due to the plant being able to improve the availability of soil nutrients, nitrogen fixation, indirectly control some weeds and soil pests (Heuert *et al*., 2021).

Just paying attention to the history of the area where the crop was introduced, because some pesticides used in sugarcane have a residual effect on the soil that may imply in the development of the crop, such as 2.4 D + Picloram, which is a of the most used active ingredients to control weeds in sugarcane (Ferreira *et al*., 2021).

2,4D + Picloram is a product with a high selectivity for monocotyledonous plants and a good effectiveness in the control of post-emergence dicotyledons and in some cases also used in the control of pre-emergence. Due to its high level of control and being one of the products with the best values available, it is widely used in sugarcane cultivation. Even though the herbicide is a contact product that acts most often in post-emergence, it ends up leaving residues of its molecules in the soil for a certain period, and when this grace period is not respected, a species of legume is introduced into the soil that has tolerance to its molecule ends up interfering with the development of the culture introduced into the soil, showing signs of toxicity in the plant, which directly impacts its productivity (Marques *et al*., 2021).

The objective of this research was to know the initial development of peanut seedlings grown in soil with herbicide residue.

2. Material and Methods

The experiment was carried out in August 2022, at Faculdades Integradas Stella Maris (FISMA), located in the Municipality of Andradina, State of São Paulo. The design was completely randomized (DIC), with five doses of the commercial product Dontor® (Picloram Acid and 2,4D Acid) as follows: absence; 1.0; 2.0; 4.0 and 8.0 L ha^{-1} and with four replications, totaling 20 plots or pots.

The pots had a volumetric capacity of 12 dm-3 and were filled with soil originating from the 0-0.3 m layer classified as Hypoferric Red Latosol (Embrapa, 2013) and had the following chemical attributes as shown in Table 1.

Table 1 - Soil chemical attributes at the time of installation of the experiment.

pH	OM	P	K	Ca	Mg	H+Al	Al	SB	CTC	V%	m%
$CaCl_2$	g dm^{-3}	mg dm^{-3}		---------------------------		mmol$_c$ dm^{-3}		---------------------------			
3,9	18	2	1,6	5	3	42	11	9,6	51,6	19	53

OM: organic matter; SB: Sum of bases; V%: Base saturation; m%: Saturation by aluminum. Source: Authors.

The soil was fertilized according to the requirements of the peanut crop according to Raij et al. (1996). And five seeds were sown five centimeters deep. During the experiment, all pots were irrigated until they reached field capacity and all cultural treatments were performed.

At 30 days after planting, the following variables were determined: plant height (PH) determined using a ruler graduated in millimeters; number of leaves (NL) determined through direct counting on the plant; total leaf area (LA) obtained using the Easy Leaf Area® application (Easlon & Bloom, 2014); shoot dry mass (SDM) and root dry mass (RDM), determined by drying in a circulation oven and air exchange at a constant temperature of 65°C until reaching a constant weight.

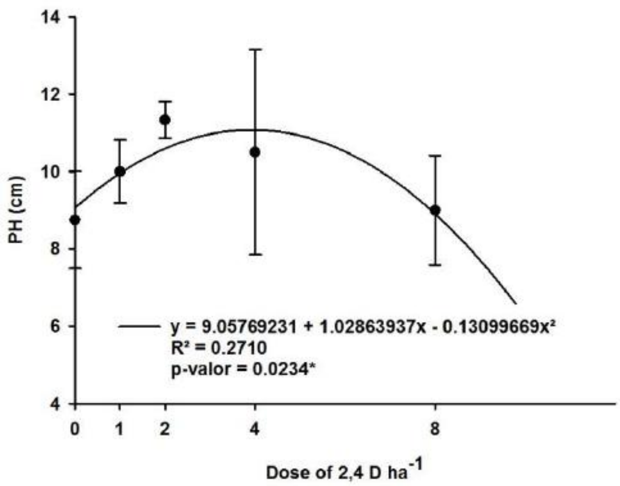
The levels of chlorophyll a and b (Chloro A and Chloro B - µmol m^{-2}) were determined by reading using the chlorofiLOG device, Falker® brand, given the values of the SPAD index (Parry *et al*., 2014) and subsequently converted in absolute values of the pigments as described by Chang and Troughton (1972) and the N-org expressed in dag/kg of dry mass was also estimated according to Ferreira (2006). Collected using cyanoacrylate ester, for the determination of stomatal functionality (FUNE) and stomatal density (DEN) (Carlquist, 1975; Castro *et al*., 2009). For all variables, five measurements were performed per slide. The plots were represented by the average value obtained from the measurements of each characteristic.

All variables were submitted to the F test ($p<0.05$) and regression analysis was applied for herbicide doses, where their models were tested: linear; quadratic and cubic (Banzatto and Kronka, 2013), where the RStudio statistical program was used (R Studio Team, 2019).

3. Results and Discussion

Peanuts responded in a positive quadratic manner to the herbicide doses present in the soil, for the height of plants, where the maximum point was approximately 4.0 liters per hectare, as shown in Figure 1.

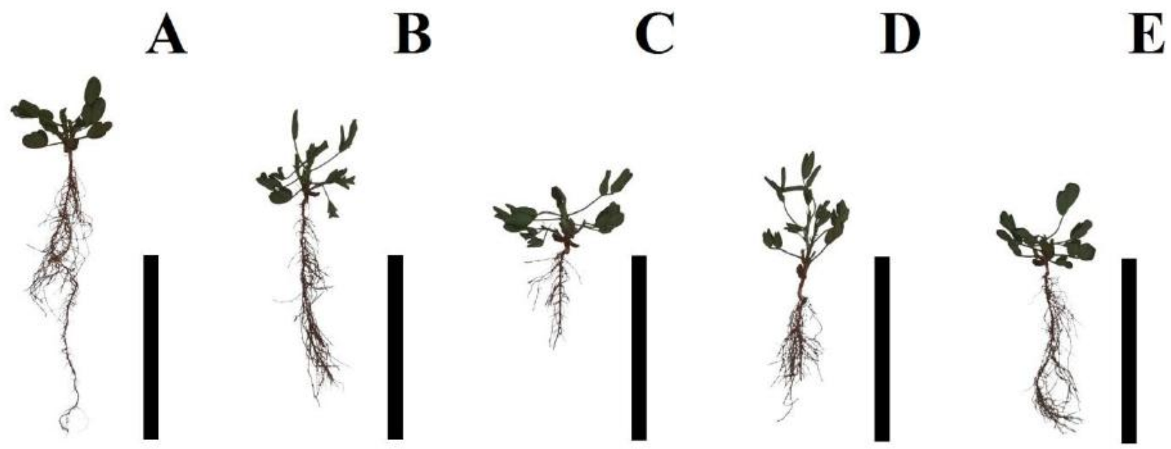
Figure 1 - Regression analysis of plant height (PH) of peanut after cultivation in soil with herbicide residue.



Source: Authors.

A positive response in plant height was not expected, due to the active principle of the herbicide being used to control eudicotyledonous plants. But there was a stimulation of the synthetic auxin in the aerial part, because when it was applied to the soil, there may have been a dilution of its concentration in the liquid part, therefore, doses above four liters of the product started to reduce the height of the plant (Gazola *et al*., 2019; Pinheiro et al., 2021), However, some plant species can metabolize the 2,4D molecule more efficiently, and thus this molecule is transformed into an original plant molecule. Children produced during auxin synthesis in sensitive eudicots and tolerant monocots showed close responses, but the amount of metabolites produced may vary, and thus results in a lower concentration of tolerant monocots compared to eudicots (Nascimento *et al*., 2020), where this difference in plant height can be demonstrated in Figure 2.

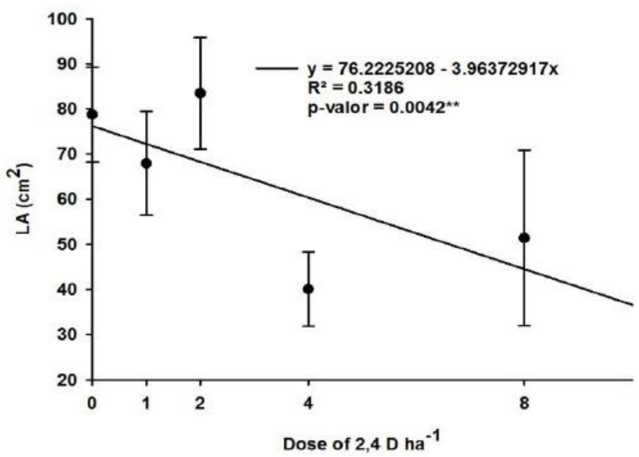
Figure 2 - Peanut plants grown in soil with herbicide residues. Bar = 10 cm.



Source: Authors.

No statistical difference was observed for the leaf area (LA) of peanuts after cultivation in soil with herbicide residue, where the overall average was 8.85. However, for leaf area, a negative linear response was observed, which resulted in a decrease in the leaf area of the plant as the herbicide doses applied to the soil increased, as seen in Figure 3.

Figure 3 - Regression analysis of peanut leaf area (LA) after cultivation in soil with herbicide residue

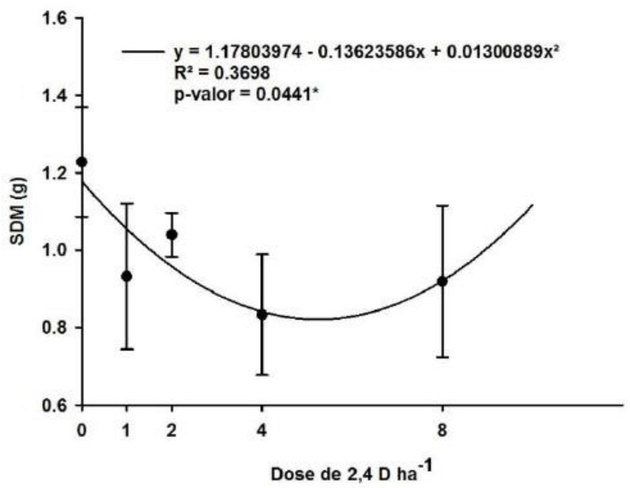


Source: Authors.

Thus, as the doses of the active principle of the herbicide in the soil increased, there was an increase in the concentration of the residue, which reflected in greater toxicity in the plant, therefore, influenced a smaller development of the leaf area. Due to the 2,4D molecule having an action similar to natural auxins, and when applied in high doses it causes a hormonal imbalance in the cells which leads to the death of the plant (Silva *et al*., 2019). When the plant has lower levels of auxins and the insufficient supply of carbohydrates in cell division and expansion, they cause the smaller size of the leaves of plants grown under shading, this implies that the photosynthesis factor restricts the availability of sugar, and combined with auxin. It is worth noting that auxins at high levels start to cause restriction in the size of the leaves due to the herbicidal action of the molecule (Gao *et al*., 2021).

Peanuts showed a quadratic response, for the shoot dry mass (SDM), where the point of maximum negative effect was 5.23 liters per hectare of herbicide applied to the soil, as shown in Figure 4.

Figure 4 - Regression analysis of shoot dry mass (SDM) of peanut after cultivation in soil with herbicide residue.

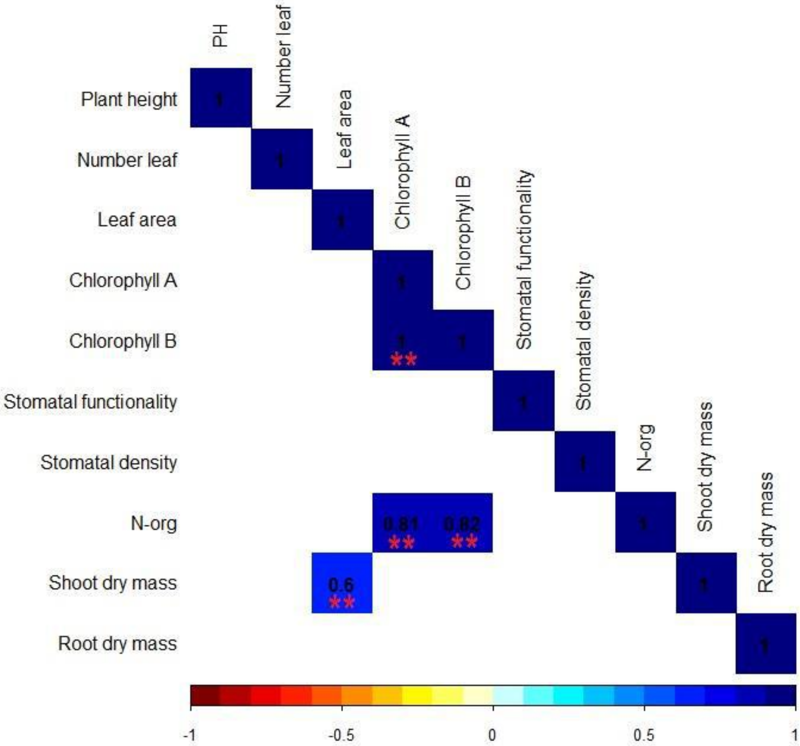


Source: Authors.

The action of the herbicide starts to cause disordered growth in the plant organism and in addition to causing hyponasty, which directly reflects on the hormonal system, preventing the natural development of plants (Silva *et al*., 2019). Plant species respond differently to herbicide residue, this response is directly linked to the molecule used, the concentration found in the residue and even the rest time after the herbicide application, and thus, the plants cannot present a good development of its dry mass (Mehdizadeh, 2019).

The dry mass presents a significant correlation with the leaf area, as shown in Figure 5, because when the leaf area increases, the plant starts to fix more carbon dioxide in its dry mass, thus the use of defoliant herbicides as in soybean and cotton it may reflect on the productivity of the crop if the use of this herbicide is applied at the not recommended time.

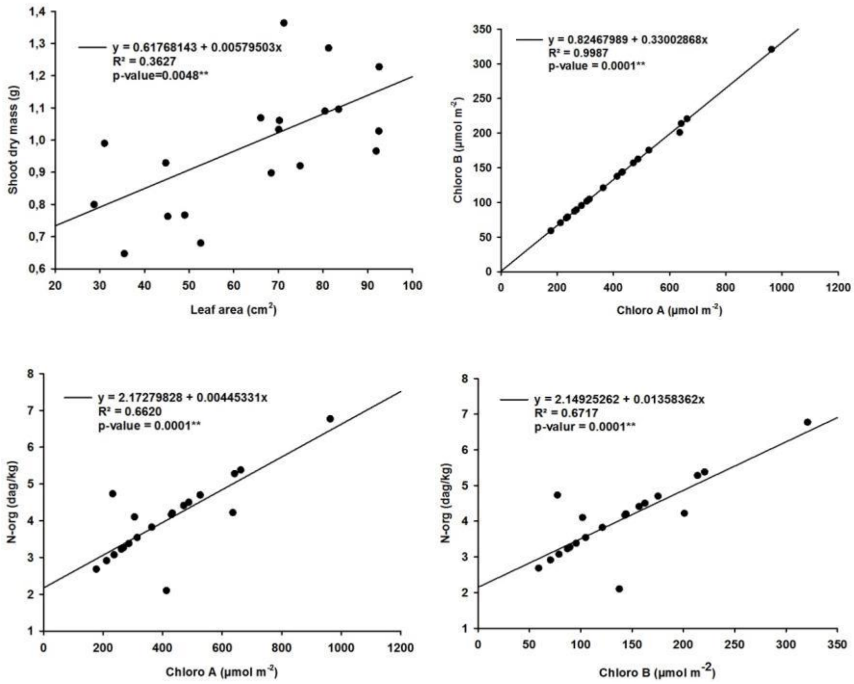
Figure 5 - Significant Pearson correlations between the analyzed variables in peanut after being cultivated in soil with 2,4D herbicide residue. ** - p <0.01.



Source: Authors.

Figure 6 shows the significant linear regressions after Pearson's correlation analysis.

Figure 6 - Significant linear regressions after Pearson's correlation analysis.



Source: Authors.

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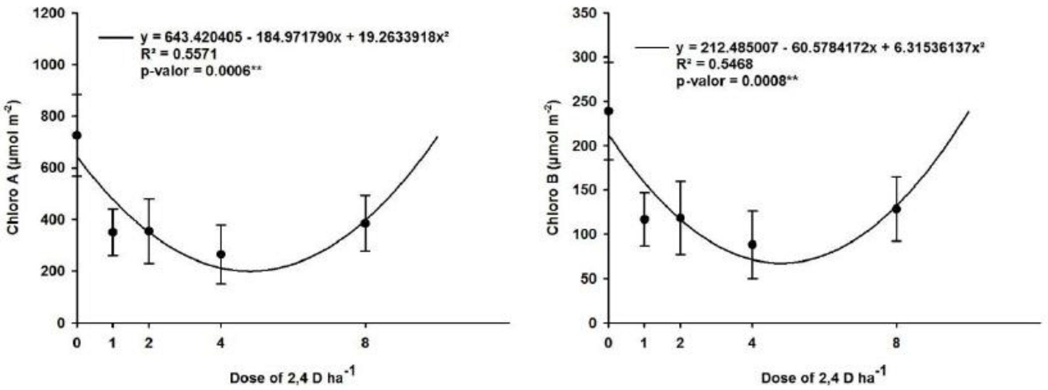
No statistical difference was observed for root dry mass (RDM) of peanuts after cultivation in soil with herbicide residue, which presented an overall average of 0.44g. Peanuts showed a quadratic response for the chlorophyll index A and B, where both showed the minimum point of 4.80 liters per hectare of herbicide applied to the soil, as shown in Figure 7.

Figure 7 - Regression analysis of chlorophyll A and B (ChloroA and B) in peanut after cultivation in soil with herbicide residue.

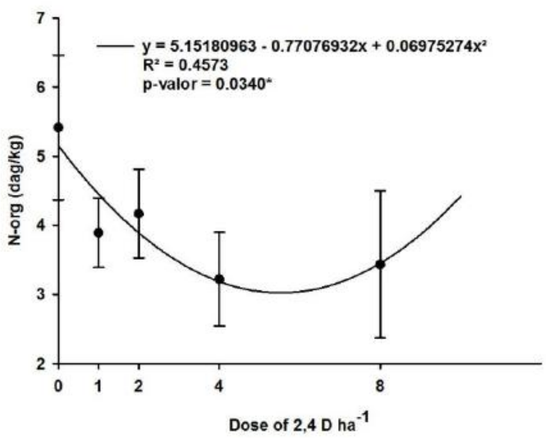


Source: Authors.

When the plant was exposed to the residue of 2,4D and picloram in the soil, the synthesis of chlorophylls was affected due to the herbicidal action that occurred on these biomolecules, since in high doses it can cause the degradation of this pigment (Kaur & Kaur, 2019). This result was expected, because with the hormonal disruption caused by the herbicide, the metabolism of sugars originating from photosynthesis was compromised, so the availability of carbon skeletons provided by sugar molecules did not occur, and thus starting to restrict the biosynthesis of this pigment and also can restrict the production of proteins for the functioning of plant metabolism (Gao *et al*., 2021; Taiz *et al*., 2017).

The peanut showed a negative quadratic response for the organic nitrogen index (N-org) where it reached the minimum point of at a dose of 5.52 liters of herbicide per hectare, as shown in Figure 8.

Figura 8 - Análise de regressão do nitrogênio orgânico (N-org) do amendoim após o cultivo em solo com resíduo com herbicida.

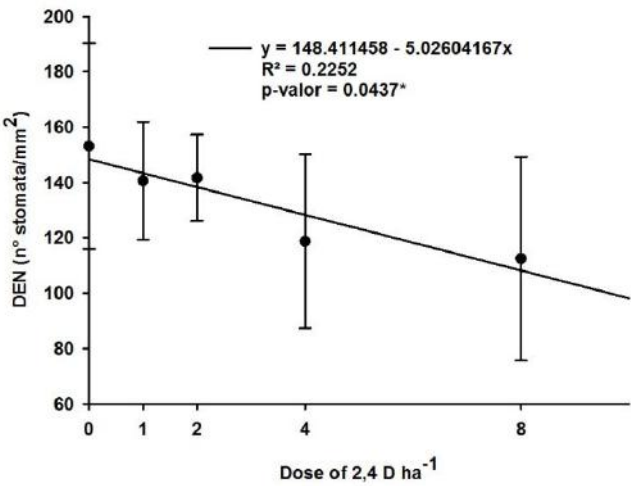


Source: Authors.

Organic nitrogen is directly related to the levels of chlorophylls A and B, proven to have a significant correlation between them, according to Figure 6, therefore, as the herbicide residue had an impact on the concentration of chlorophylls A and B, and this element is part of the chemical composition of these pigments, then the concentration of organic nitrogen showed similar negative response (Kaur & Kaur, 2019). It is worth noting that nitrogen is one of the main nutrients for plants, as they begin to compose structural proteins and especially enzymes, so when nitrogen metabolism is compromised, the entire plant metabolism is altered (Taiz *et al*., 2017; Lisboa *et al*., 2022).

No statistical difference was observed for stomatal functionality (FUNE) of peanuts after cultivation in soil with herbicide residue, which presented an overall average of 0.43. Peanut showed a negative linear response in the regression analysis for stomatal density (SND), as seen in Figure 9.

Figure 9 - Regression analysis of peanut stomatal density (DEN) after cultivation in soil with herbicide residue.



Source: Authors.

The residue left by the herbicide in the soil implies a toxicity in the plant, in this way, the action of high concentrations results in injuries and leaf necrosis, this phenomenon reflects negatively on the development of the plant, due to the impairment in the multiplication of its cells and also in their differentiation in the stomatal complex, starting to reflect in the number of stomata and this negative response in their functionality was also expected (Pinheiro *et al*., 2021). Studies show that the high correlation between stomatal density and plant dry mass, as reported by Neves *et al*., (2022), may have compromised CO_2 fixation in peanut dry mass.

4. Conclusions

The maximum point in the peanut response was at the dose of 5.0 liters per hectare of the herbicide 2,4D + Picloram.

A dose greater than 5.00 liters per hectare of the herbicide 2,4D + Picloram compromises the development of peanuts.

Leaf area and stomatal density responded in a negative linear fashion to herbicide doses.

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