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Eucalyptus shading and allelopathy in the germination and development of pitaya (*Hylocereusundatus)*

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Abstract

Eucalyptus spp. is known to be part of most forests planted for commercial use in Brazil. However, when intercropped with Pitaya (*Hylocereusundatus*), few studies report this interaction. The objective of this studywas to evaluate the effects of shading and the existence of allelopathic interactions between *Eucalyptus* spp. and pitaya (*H. undatus*) in the field, as well as the action of eucalyptus leaf extract in the germination of pitaya seeds underlaboratory conditions. In the field experiment, a randomized block design (DBC) was used, with the use of adult pitaya and eucalyptusplants, evaluating the following variables: number of sprouts in the shoot; number of flowers; crown diameter; circumference of the cladode; number of fruits and variation in the amount of light reaching the plants. It was observed that the plants outside the eucalyptus forest showed better results. In the second experiment, the allelopathic action of different concentrations of aqueous extracts of eucalyptus leaves on the germination of pitaya seeds was evaluated. This experiment was carried out in a CRDwith four replications of 60 pitaya seeds. These seeds were then subjected to treatments with different dilutions, varying from 0% (control), 12.5%, 25% and 50% of the stock solution. It was observed that, as the leaf extract concentrations increased, germination decreased. The concentrations 0% (control) and 12.5% showed better results. In conclusion, eucalyptus showedan allelopathic action on pitaya plants.

Keywords: Allelochemicals, Phytotoxicity, *Eucalyptus* spp, Viability

Introduction

The population has been looking for a balanced, varied and nutrient-rich diet, which can be fully served, not only by the fruits traditionally produced on a large scale, but also by those considered to be exotic.

Among exotic fruits, pitaya has stood out due to its high nutritional and antioxidant potential (Chen et al., 2019), its fruits are rich in phenolic compounds, betalains (García-Cruz et al., 2013), vitamins and minerals such as: potassium, iron, calcium, manganese and zinc, (Cordeiro et al., 2015).

In nature, pitaya is found in shaded environments of tropical forests in America in understory conditions, under irradiance of 500 µmol m^{-2} s^{-1}, considered to be low light radiation (Almeida, 2016).

An option for shading is the planting of pitaya intercropped with larger trees that lead to this condition. The shading effect involves multiple mechanisms, such as a better system for removing oxidants in plants and a greater accumulation of protectors against oxidation. Other favored characteristics are: water balance, greater carbon fixation, reduction in energy expenditure in transpiration and respirarion, besides reduction inphotodamage (Pio et al., 2020).

Intercropping can lead to gains in yield, better use of natural resources, making agricultural systems more sustainable (Silva et al., 2020), enabling the maximization of use of environmental resources in different crops, resulting in a better land use (Guedes et al., 2010).

Eucalyptus is widely used as a windbreak in orchards. However, its function when intercropped with fruit trees or even live posts can be quite useful, especially in pitaya, which is a plant that needs support for its development.

However, it is known that eucalyptus has allelopathic activity on several species, due to the

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presence of organic compounds released by the roots or leaves that are sources of essential oils with a wide biological activity, including fungicide, insecticide, herbicide and acaricide (Batish et al., 2008), which can inhibit the development of other plants. However, studies on fruit trees intercropped with *Eucalyptus* spp. are still scarce in the literature, and most of them have been conducted under laboratory conditions.

This experiment was designed to observe the effect of eucalyptus shading and allelopathy on pitaya plants in the field. However, it would not be possible to distinguish the effect of shading and allelopathy in a single experiment. Therefore, the idea of germination of pitaya seeds with eucalyptus leaf extracts could elucidate the problem of allelopathy. Thus, this study aimed to evaluate the effects of shading and the existence of allelopathic interactions between *Eucalyptus* spp. and pitaya (*H. undatus*) in the field, as well as the possible allelopathic action of eucalyptus leaf extracts in the germination of pitaya seeds under laboratory conditions.

Material and Methods

Two experiments were carried out, one in the field using adult plants of pitaya and eucalyptus and the other in the laboratory, with the use of pitaya seeds and eucalyptus leaf extract.

The field experiment was carried out on a property, in the municipality of Ingaí-MG, Latitude: 21° 24' 24'' South, Longitude: 44° 56' 30'' West. The experiment area has a slightly undulating slope, located in the Mesoregion of Campo das Vertentes in Minas Gerais; the climatic classification of the region is Cwa, according to the Köppen classification.

Eucalyptus was planted in 2012. When the trees reached four years of age, in January 2016, then approximately 15 m tall, the pitaia orchard was set inside and in front of the eucalyptus grove, with a standardized plant height of 1.8 meters for all treatments, without using an irrigation system. Seedling planting started from the third row (L3) of eucalyptus until 11 rows (L11) were completed, and row 11 was considered as the border. Rows 1, 2 and 3 are inside the eucalyptus grove, while rows 4 to 10 are in the open field, where rows 4 to 7 were partially shaded throughout the day and the other rows were in full sun.

Inside the forest, eucalyptus trees were used as live posts for the management of pitaya plants. For plants outside the forest, they were conducted in 1.8-m high eucalyptus posts, treated with boric acid, potassium permanganate and copper sulfate. The spacing adopted for pitaya followed the same spacing as that of eucalyptus, 4 m between rows and 4 m between plants, both for plants inside and outside the forest.

Before the implementation of the experiment, chemical analysis of the soil was carried out at a depth of 0-20 cm. The chemical attributes of the soil, as well as contents of macro- and micronutrients, are presented below: pH in water (5.3); K=126.47, P=5.89mg/dm^3; Ca=2.27, Mg=1.07, Al=0.10cmolc/dm^3; V%=46.62; M.O=2.67dag/Kg; Zn=2.10, Fe=35.10, Mn=9.70, Cu=1.10, B=0.07, S=5.10mg/dm^3.

For the implantation of the pitaya orchard, 50x50x50-cm pits were made and, in each pit, 10 L of tanned pen manure, simple superphosphate (P_2O_5) and limestone ($CaCO_3$) were placed for soil correction; cover fertilization was carried out with NPK 10-10-10 (50g/plant) 30, 60, and 90 days after planting. Cover was carried out in the first year with 3L of quail manure per plant in October, December and February.

The driving system adopted was that of a pod, with a single seedling/pod driven on a single stem, forming a sprout crown at the top of the posts. The pitaya plants in the live posts were pruned at 1.80m height, in the same way as the others.

In January 2018, evaluations of the following variables started: a) number of sprouts in the shoot; b) number of flowers; c) crown diameter: considering the two largest branches on opposite sides in the shoot of each plant, with the aid of a measuring tape; d) circumference of each cladode of plants at 10 cm in height from the soil, using a digital caliper and e) number of fruits.

To check the variation in the amount of light reaching the area, a point was selected on each row and a lux meter was used for measures, at 10, 12 and 15 o'clock, of the light gradient along the rows. In the places where the solar radiation arrived without being intercepted by the trees and there was maximum illumination, the value of 100% was considered and, in the places where there was some kind of interference and/or interception of light, the values were relativized (Figure 1).

A randomized block design (RBD) was used, with 3 blocks of 4 plants of each treatment per block. Data were subjected to analysis of variance, considering 5% as the level of significance, with a square root transformation on the variables number of flowers and fruits, since they are counting data. For the variable number of sprouts, even if counting, it was not necessary to make the transformation, since no assumption of the analysis of variance was violated. The Tukey test was also used at 5%.

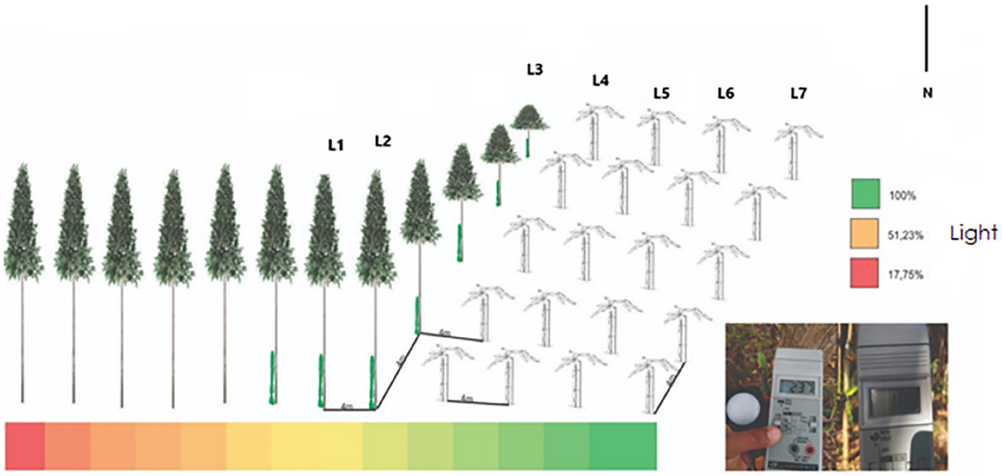

Figure 1. Radiation gradient inside and outside the woodlands. Measurement of the amount of light incident in the lower part of the forest with the use of a luximeter, in which the red color indicates the places of greatest radiation, while the green color indicates the lowest index of solar radiation arriving.

The seed germination experiment was carried out at the Pomology Laboratory in the Fruit Sector of Federal University of Lavras. The pitaya seeds were extracted manually from the ripe fruits of better appearance as color, size and brightness. The pulp mucilage adhered to the seeds was removed using a 2-mm steel sieve in running water using lime until the seeds were completely cleaned, adapted according to the method proposed by Gentil and Ferreira (2000). The seeds were then distributed in a plastic container and left at room temperature for drying.

For the preparation of the aqueous extract, the eucalyptus leaves were collected under the trees at the site of the field experiment and taken to the laboratory, where they were dried in a forced flow chamber at 50°C until they reached constant weight. Subsequently, the leaves were crushed in a blender and passed through a 0.5-mm sieve to remove larger particles, resulting in a homogeneous powder.

A 1:9 stock solution was prepared using 100 g of the eucalyptus leaf extract diluted in 900 mL of distilled water, to reach a volume of 1 L. This solution was left to stand at room temperature for 72 hours; the material was then filtered twice, one filtration using a 0.5-mm plastic sieve to remove larger particles and the other using filter paper so that, at the end of the process, only the homogeneous extract of the eucalyptus leaves could be obtained.

The filtration product was considered as the stock solution, rich in allelopathic compounds, defined as a 100% concentrate from which the aliquots for the different treatments applied to the pitaya seeds were extracted.

The germination test was carried out according to the methodology of (Alves et al., 2011), differentiating only the number of evaluations, changing from two to three.

The seeds were kept in Gerbox boxes in B.O.D with a constant temperature of 25° and a 12-hours photoperiod, according to the methodology of Alves et al. (2011). The evaluations were carried out at 5, 10 and 15 days after sowing. The seeds that emitted radicle and/or shoot were considered as germinated. The germination values for each treatment were expressed as a percentage.

The Germitest paper were weighed on a precision scale and then moistened with the aliquots of the selected test solution, in order to achieve a ratio of 2.5 times the weight of the paper.

The model used in the experiment was the Completely Randomized Design (CRD), with four treatments that consisted of different extract dilutions (the control treatment was T0 = 0% (consisting only of distilled water), T1 = 12.5%, T2 = 25% and T3 = 50% of the stock solution) and four replications of 60 seeds in Gerbox plastic boxes on two sheets of Germitest paper, analysis of variance at 5% significance and the means referring to the collected data were compared with each other by the Tukey test also at 5% significance.

Results and Discussion

In the field experiment, based on the results of the statistical analysis, it was observed that the pitaya plants that were in the planting rows L4, L7 and L8, as shown in Figure 2, even presenting a greater number of sprouts on average, did not differ statistically from the others (F = 2.099 and p value approximately equal to 0.05), that is, none of the planting rows that were inside the eucalyptus forest (L1, L2 and L3) emitted enough sprouts to be considered productive plants.

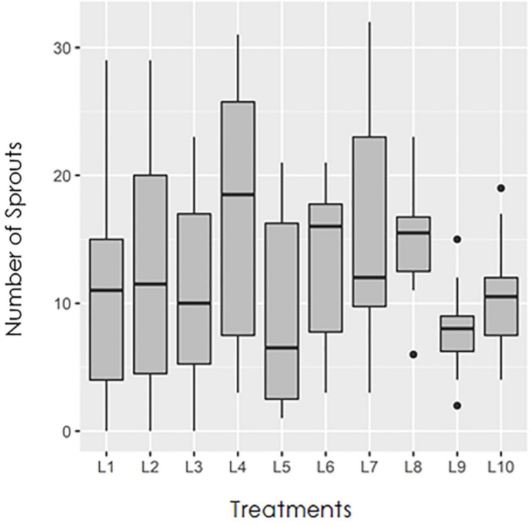

Figure 2. Number of sprouts produced per plant in each row.

Figure 3 shows the light variation at different times of the day and in relation to the plants that were under the eucalyptus forest and the plants that were in full sun. Therefore, throughout the day, the plants that were in the shading condition of the eucalyptus received a lower incidence of light, as expected, when compared to plants that were in full sun.

It is also noteworthy that the plants that were in the rows right after the eucalyptus planting (Row 4) had a reduction in the incidence of light, most likely caused by the change in eucalyptus shading throughout the day. In the year of the experiment, the solar radiation varied from 5209 wh/m^2 day in July to 3334 wh/m^2 day in November (INPE, 2021).

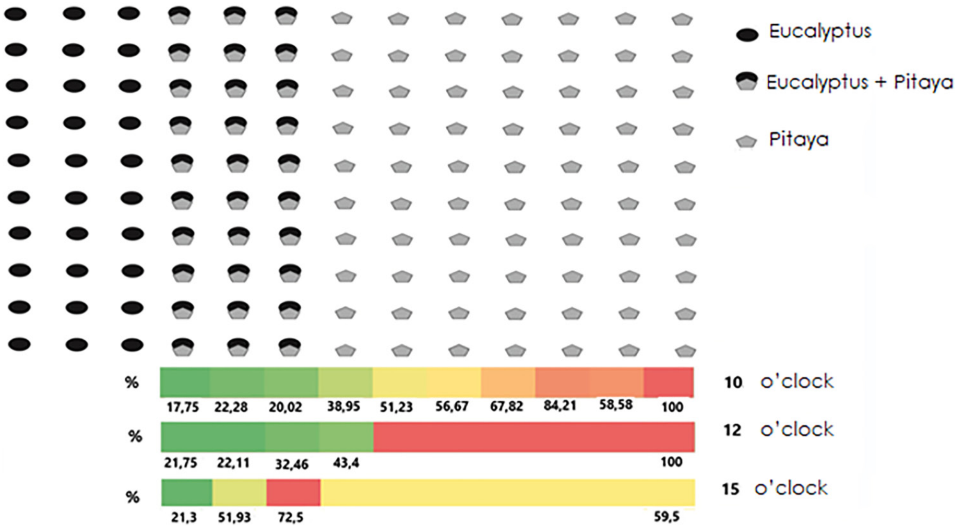

Figure 3. Planting scheme of pitaya and eucalyptus and quantification of light, at different times of the day.

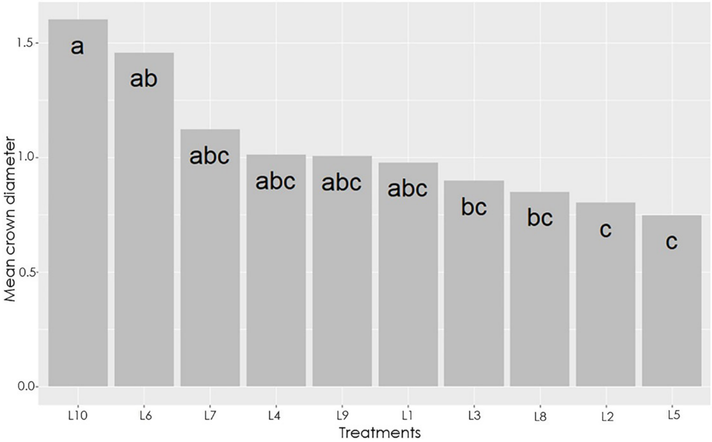

Figure 4. Mean crown diameter produced per plant in each row.

In relation to crown diameter (Figure 4), through the analysis of variance, it was found that between the different rows there was a statistical difference (F = 3.872 and p value equal to 0.000). The diameter of the cladodes at 10 cm in height did not show significant differences between the plants that were inside the woodland and under the influence of the shading of eucalyptus and those that were in full sun.

Regarding the number of flowers produced by the plants (Figure 5), it can be observed that the plants that were outside the woodland (L7, L10), even showing higher results for mean number of flowers, were equal to each other and also to L8, L6 and L9 (F = 8,180 and MSD (minimum significant difference, considering the Tukey test) = 1,498), The number of flowers and the number of fruits are two important variables to be analyzed, since the second depends directly on the first, as the number of fruits that will be produced by each plant can be indirectly deduced by the number of flowers (Figure 6).

In the case of fruits, a transformation was made (square root transformation) whose value of F = 20.743, with MSD = 0.815.

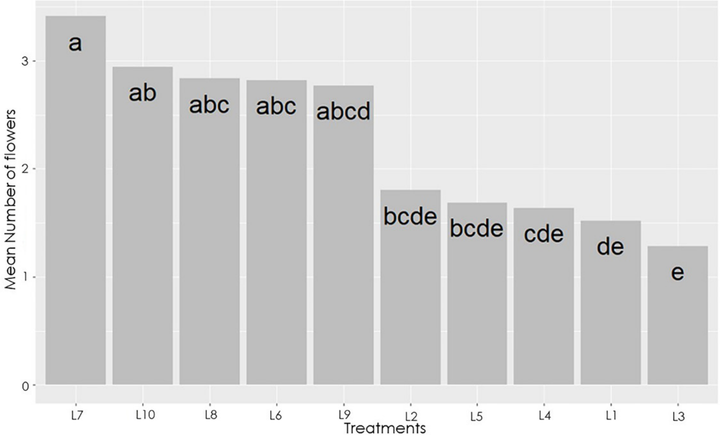

Figure 5. Flowers*produced per plant in each row. * transformed data.

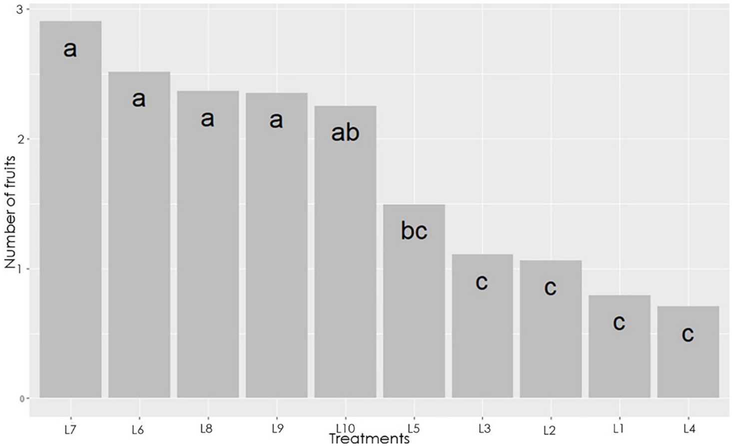

Figure6. Number of fruits produced per plant in each row; data is transformed.

Silva et al. (2015) evaluated the effects of shading with dark screens in *H. undatus* and observed that shading positively affected the production of flowers; under this condition, they had 13% more flowers than plants that were in full sun.

Chang et al. (2016) state that shading in pitaya can cause a decrease in the flow of photosynthetic photons, while shading is also effective in reducing burns (and/or yellowing) in the cladodes that are the photosynthetic areas of these plants, in the same way that shaded plants can also show bigger fruits.

According to Raveh et al. (1998), *H. polyrhizus* plants under shading of up to 60% were 2.2x more efficient in flower production than those grown under shading of 30% which, consequently, produced more than plants grown without protection, which in fact was not observed in this experiment; on the contrary, plants under full sun showed better development, probably influenced by local climatic variables.

Plants in full sun also showed better results for almost all the characteristics analyzed, except for crown and cladode diameter, as there was no statistically significant difference between plants shaded by eucalyptus compared to plants grown in full sun. This result differs from those found by Cavalcante et al. (2011), who observed a greater number of secondary branches, which would characterize a larger crown diameter, in the same way that the cladodes had diameters from 11.94% to 18.53% larger when the plants were under screens of up to 75% compared to plants exposed to direct radiation.

The results observed by Andrade et al. (2006) in the shading of *H. undatus* with high density screens showed a positive effect, differing from this study, when only the shading factor is taken into account. Despite the knowledge that direct exposure to solar radiation is harmful to *H. undatus*, plants exposed to direct radiation still performed better than those positioned close to eucalyptus plants.

It should be considered that *Eucalyptus* spp. plants may show allelopathic activity (Chu et al., 2014), either in their leaves present in the plant litter (Ahmed et al., 2018) or released from their roots (Liu et al., 2018). Therefore, the fact that eucalyptus releases compounds that may in some way influence pitaya development is already a factor to be considered as a disadvantage for the use of this species as a live post.

The genus *Eucalyptus* is known for its allelopathic action on several species of economic interest such as soybeans (Abdelmigid & Morsi, 2017), *Urochloadecumbens* and *Panicum maximum* (Carvalho et al., 2015). These deleterious effects for other plants, according to Zhiqun et al. (2017), is due to the presence of organic compounds such as monoterpenes released by several *Eucalyptus* spp. species, one of the genera most used commercially in planted forests in the world, including in Brazil.

In order to know a little more about the allelopathic action of eucalyptus, a second experiment was carried out in order to observe the possible phytotoxic effects of the eucalyptus leaf extract solution on the germination of pitaya seeds.

Table 1 shows the comparison of means; the three treatments containing eucalyptus leaves showed significant inhibitory effects on the germination of pitaya seeds and, as the concentration of the aqueous extract increased, it was possible to observe a decrease in seed germination of the seeds. seeds.

Table 1. Seed germination as a function of the addition of aqueous extract of eucalyptus leaves.

Aqueous extract (%)	Germination (%)
0	65.0a
12.5	47.5b
25	36.25 c
50	8.0d
CV %	12.60

Means followed by the same lowercase letter in the column, do not differ by the Tukey test at 5% probability. (T0: control; T1: 12.5; T2: 25 and T3: 50% aqueous eucalyptus extract).

Treatment T0, in which the seeds were submitted only to the addition of water, germination was higher than that of the treatments that received the doses of the extract. In treatment T3, in which the seeds received a dose of 50% of the eucalyptus extract, there was greater inhibition of germination. Therefore, seeds submitted to treatment 1 obtained a germination rate of 70.07% in relation to the control, to treatment 2, of 55.76 germination, and finally to treatment 3, presenting a germination rate of only 12.30% in relation to the control.

It is possible to observe that in all treatments that received some dose of eucalyptus extract, germination was inhibited at some level. That is, the compounds in the eucalyptus extract somehow inhibited seed germination, even at the lowest concentration of 12.5%.

These results are, therefore, similar to those found by Carvalho et al. (2015), who analyzed the inhibitory effects of *Eucalyptus urograndis* leaf extracts on forage grass seeds and observed that, in fact, as the concentrations of eucalyptus extracts increased, the percentage of germinated seeds decreased significantly, as well as an increase in the number of abnormal seedlings was observed.

The same authors concluded in their study that, at higher concentrations, there is a greater number of seedlings with oxidized roots, a fact that was also observed in this study, in which newly germinated seeds that were treated with 12.5 and 25% of extract presented an oxidized radicle.

This may indicate, therefore, that, besides inhibiting seed germination, the allelopathic compounds in the extracts can cause changes in plant growth and initial development, since their root system is damaged, it will have difficulties in developing normally, in addition to effects on primary metabolism, highlighting changes in membrane permeability, in DNA transcription and translation, in the functioning of secondary messengers, in respiration, in the formation of enzymes and receptors, or even in the combination of these factors (Formigheiri et al. 2018).

Still regarding the percentage of germination, the results of this experiment are consistent with those of Ferreira &Faria (2007), who observed a significant reduction in germination when the seeds were treated with ethanolic extract of *Eucalyptus citriodora* until the third day showing, therefore, a significant difference in the percentage of seeds germinated at the end of seven days.

In treatments T1 (12.5% of the aqueous eucalyptus extract) and T2 (25%), there was initially a statistical difference in germination until the tenth day; on the fifteenth day, the means had a similar behavior, while T3 (50%), when compared to the control treatment T0, was completely contrasting (Figure 7).

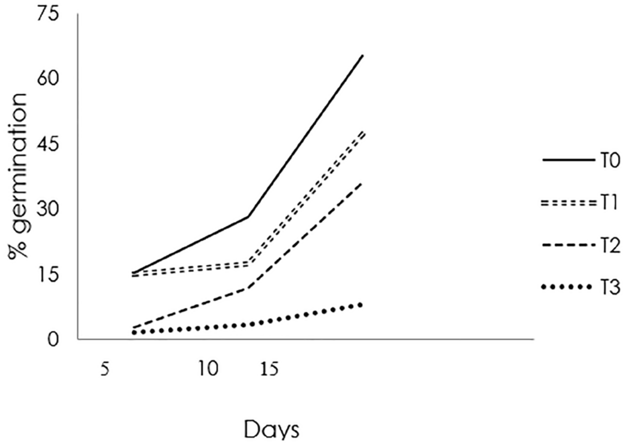

Figure 7. Seed germination over an interval of 15 days; evaluations performed at 5, 10 and 15 days after the initial treatment. (T0: control; T1: 12.5; T2: 25 and T3: 50% of the aqueous eucalyptus extract).

It is important to observe that the allelopathic potential can be widely variable between plants, and depends on factors such as species, age of plants and the stage of degradation of the material where allelopathic compounds are found (Ferreira &Faria, 2007). An example was observed by Bedin et al. (2006) who, after applying extracts of fresh and dry leaves of *Eucalyptus citriodora* on tomato seeds (*LycopersicumesculentumM.*) concluded that neither fresh nor dry leaves inhibited seed germination.However, at concentrations of 3 and 5 % of aqueous extract, germination speed was drastically influenced.

In Figure 8, it is observed that, as the concentrations of extract in the seeds are increased, there is greater inhibition in their germination, whereas in T0 (control), there is 0% inhibition of germination; in T1, there is 44.23%; in T2, 45.51 and in T3, 87.17 of inhibition. These results may be related to the concentration of allelopathic compounds present in the extracts once, as the concentrations increased, these compounds had greater bioactivity on seed germination.

Observing Figure 9, in which the highest concentrations of aqueous eucalyptus extract are on the left, it exactly coincides with the boxes where the seeds have the lowest germination rate. T3 = 50% of extract has 3 germinated seeds; in T2 = 25% of extract, 33 seeds germinated; in T1 = 12.5% of extract, 28 germinated seeds and, in T0, 40 germinated seeds were counted. It can then be concluded that, as the concentrations of the extracts decrease, there is a greater amount of germinated seeds.

These results are corroborated by those found by Ahmed et al. (2018), in which the inhibitory effects of *Eucalyptus camaldulensis* leaves on seed germination and on the development of the root system of some agricultural crops were directly related to the concentrations of leaves incorporated in the form of litter in the soil, so that the places that received higher concentrations of leaves on the seeds showed shorter root lengths and fewer shoots. For these authors, these inhibitory effects may be related to the presence of bioactive allelochemicals present in *Eucalyptus* spp. leaves, such as chlorogenic acids, alkanes, terpenes, aldehydes, phenols, among others.

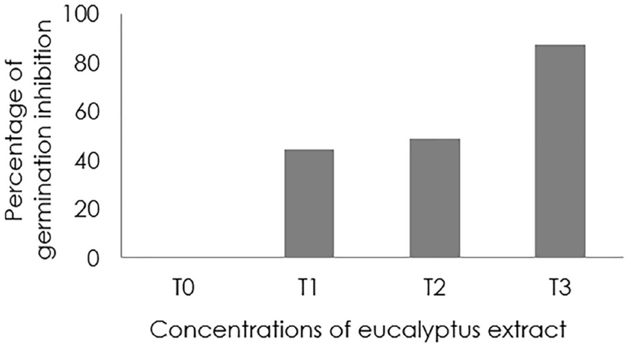

Figure 8. Percentage of germination inhibition of pitaya seeds submitted to different concentrations of eucalyptus extract.

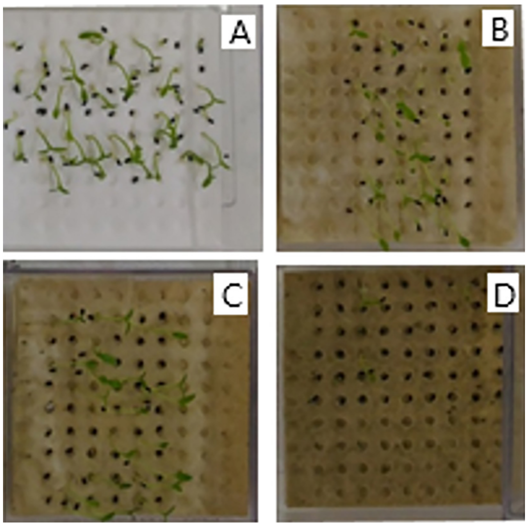

Figure 9. Germination of pitaya seeds submitted to different concentrations of eucalyptus extract (A) control; (B) 12.5% eucalyptus extract; (C) 25%and (D) 50%.

Similar results were found by Zhang et al. (2010), when using *E. grandis* root extract; they concluded that these inhibitory effects on the germination of species such as *Phaseolus aureus* were more accentuated as the concentrations of root extracts were higher; the inhibition of *P. aureus* germination reached 51.2%.

Some studies, such as that by Benchaa et al. (2018), have reported the potential of *Eucalyptus* spp. to inhibit, at very low concentrations, the germination of seeds and the growth of seedlings of several species, including the suppression of seed germination of the field mustard (*Sinapisarvensis*), which can reach 100%. The effects of these compounds can even play a role as a herbicide in seedlings with up to four pairs of leaves.

To test the possible herbicidal effects of *Eucalyptus globulus*Labill on lettuce seeds (*Lactuca sativa* L., ‘Grandes Lagos’) and *Agrostis stolonifera* L., ‘Penncross’, Puig et al. (2018) used aqueous extracts obtained from leaves and it was found that these extracts also had significant effects on both root germination and length. The same authors found that, in addition to having effects in the early stages of seedling germination, the aqueous extracts of *E. globulus* were phytotoxic in adult plants in order to directly interfere in their development.

Bonanomi et al. (2005) worked with several species, and observed that the inhibition of germination, growth and development is directly related to the degree and time of decomposition, the plant material (leaf or root) and mainly the species involved in the allelopathic relationship, as some species, however close they may be, present very different degrees of phytotoxicity; in this case, it cannot be generalized (stating) that species that, even though of the same genus, present the same allelopathic dynamics.

Conclusions

Shading, associated with the allelopathy caused by the eucalyptus plants, impairs the development of pitaya plants, influencing mainly on the number of flowers and fruits produced.

Aqueous extracts of eucalyptus leaves have inhibitory activity in the germination of pitaya seeds and, with the use of 50% eucalyptus extract, there was greater inhibition of germination.

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