



CHEMICAL PROFILE AND ALLELOPATHIC POTENTIAL OF *ASPARAGUS SETACEUS* (KUNTH) JESSOP (ASPARAGACEAE)

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ABSTRACT

Objective: This work aimed to elucidate the chemical profile and the allelopathic potential of the ethanolic extract of *Asparagus setaceus* leaves collected in the morning (8 A.M) and afternoon (2 P.M).

Method: The extract was obtained using cold ethanol and the secondary metabolites classes were identified with specific reagents. For allelopathic activity, the test solution was obtained from 100 mg of ethanolic extract, dissolved in 100 mL of 66% ethanol, obtaining concentrations from 6.25% to 100%. Two control groups were used: distilled water and 66% ethanol alcohol in the evaluation of the following parameters: germination percentage (GP), germination speed index (GSI) and stem and radicle length.

Results and Discussion: The tabulated results were analyzed by two-way ANOVA, through analysis of variance and Tukey's test. The pH values of *Asparagus setaceus* ethanolic extract in the morning ranged from 9 to 12, for

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Asparagus setaceus ethanolic extract in the afternoon ranged from 8 to 12. In the phytochemical analysis, the morning extract showed a greater number of metabolites when compared to the same extract prepared in the afternoon, causing greater negative interference observed in the germination speed index and in the development of seedlings.

Research Implications: Therefore, the combined action of these compounds and factors such as humidity, temperature and soil pattern should be considered when evaluating the different results found, and further studies are needed to investigate the specific constituents of allelopathic activity.

Keywords: Allelopathic Activity, Secondary Metabolites, Germination, *Asparagus setaceus*.

PERFIL QUÍMICO E POTENCIAL ALELOPÁTICO DE FOLHAS DE *Asparagus setaceus* (Kunth) Jessop (Asparagaceae)

RESUMO

Objetivo: Este trabalho teve como objetivo elucidar o perfil químico e o potencial alelopático do extrato etanólico de folhas de *Asparagus setaceus* coletadas no período da manhã (8h) e da tarde (14h).

Método: O extrato foi obtido utilizando etanol frio e as classes de metabólitos secundários foram identificadas com reagentes específicos. Para atividade alelopática, a solução teste foi obtida a partir de 100 mg de extrato etanólico, dissolvido em 100 mL de etanol 66%, obtendo concentrações de 6,25% a 100%. Foram utilizados dois grupos controle: água destilada e álcool etílico 66% na avaliação dos seguintes parâmetros: porcentagem de germinação (PG), índice de velocidade de germinação (IVG) e comprimento de caule e radícula.

Resultados e Discussão: Os resultados tabulados foram analisados por ANOVA a dois critérios, através de análise de variância e teste de Tukey. Os valores de pH do extrato etanólico de *Asparagus setaceus* no período da manhã variaram de 9 a 12, para o extrato etanólico de *Asparagus setaceus* no período da tarde variaram de 8 a 12. Na análise fitoquímica, o extrato da manhã apresentou maior número de metabólitos quando comparado ao mesmo extrato preparado no período da tarde, causando maior interferência negativa observada no índice de velocidade de germinação e no desenvolvimento das plântulas.

Implicações da Pesquisa: Portanto, a ação combinada desses compostos e fatores como umidade, temperatura e padrão do solo devem ser considerados na avaliação dos diferentes resultados encontrados, sendo necessários mais estudos para investigar os constituintes específicos da atividade alelopática.

Palavras-chave: Atividade Alelopática, Metabólitos Secundários, Germinação, *Asparagus setaceus*.

PERFIL QUÍMICO Y POTENCIAL ALELOPÁTICO DE HOJAS DE *Asparagus setaceus* (Kunth) Jessop (Asparagaceae)

RESUMEN

Objetivo: Este trabajo tuvo como objetivo dilucidar el perfil químico y el potencial alelopático del extracto etanólico de hojas de *Asparagus setaceus* recolectadas en la mañana (8 am) y la tarde (2 pm).

Método: El extracto se obtuvo utilizando etanol frío y las clases de metabolitos secundarios se identificaron con reactivos específicos. Para la actividad alelopática, la solución de prueba se obtuvo a partir de 100 mg de extracto etanólico, disueltos en 100 mL de etanol al 66%, obteniendo concentraciones de 6,25% a 100%. Se utilizaron dos grupos de control: agua destilada y alcohol etílico al 66% para evaluar los siguientes parámetros: porcentaje de germinación (PG), índice de velocidad de germinación (IVG) y longitud de tallo y radícula.

Resultados y Discusión: Los resultados tabulados fueron analizados mediante ANOVA de dos vías, mediante análisis de varianza y prueba de Tukey. Los valores de pH del extracto etanólico de *Asparagus setaceus* de la mañana oscilaron entre 9 y 12, para el extracto etanólico de *Asparagus setaceus* de la tarde oscilaron entre 8 y 12. En el análisis fitoquímico el extracto de la mañana mostró un mayor número de metabolitos en comparación con el mismo extracto preparado en la tarde, provocando mayor interferencia negativa observada en el índice de velocidad de germinación y desarrollo de las plántulas.



Implicaciones de la investigación: Por lo tanto, se debe considerar la acción combinada de estos compuestos y factores como la humedad, la temperatura y el patrón del suelo al evaluar los diferentes resultados encontrados, y se necesitan más estudios para investigar los constituyentes específicos de la actividad alelopática.

Palabras clave: Actividad Alelopática, Metabolitos Secundarios, Germinación, *Asparagus setaceus*.

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1 INTRODUCTION

Allelopathy is described as a mechanism by which living or dead plants release chemical compounds capable of negatively or positively interfering with the growth and development of other plants. In nature, this activity plays an important role in the formation of plant communities, germination, plant succession and domination of environments. This is the strategy commonly used by exotic plants to colonize natural environments (Campos et al., 2022).

Chemical compounds such as phenols, terpenes, alkaloids and flavonoids come from different plant organs and when released into the environment act as allelochemicals. These can mediate biochemical interactions between plants and are released through exudation, volatilization, leaching and mainly by the decomposition of plant remains (Silva et al., 2021).

The action of these compounds can prevent cell division, elongation processes, germination and plant growth. They normally act by modifying the synthesis of the main components that form plants, altering the distribution of carbons in cells, preventing regulation and growth hormones and accelerating or reducing the activities of plant metabolism enzymes (Rodrigues et al., 2020).

As a more advantageous alternative, allelochemicals are being widely used as natural herbicides. Unlike conventional methods, these reduce the risk of environmental contamination, limit changes in invasive species and reduce resistance to existing compounds on the market. In this sense, allelochemicals can contribute as a basis for the synthesis of agrochemicals that are less harmful to health and biodiversity (Machado; Muniz; Terra, 2021).

In the search for control alternatives, it was observed that many plant extracts present activities of biological interest that contribute to the development of sustainable agriculture. The extracts demonstrate antimicrobial, antioxidant, insecticide and allelopathic activities and can act in the control of pests and plant diseases with less toxicity and environmental impact (Silveira et al., 2021).



These characteristics are observed in plants of the *Asparagaceae* family, such as *Asparagus setaceus* (Kunt), popularly known as “melindro”. Species of this family have numerous phenolic compounds, terpenes, and ascorbic acid, thus justifying their biological activities. The allelopathic action occurs due to the presence of lipophilic compounds of high solubility and phenols, as these have degenerative effects on cell membranes (Hesham et al., 2012). Therefore, the present study aimed to elucidate the chemical profile and the allelopathic potential of the Ethanolic Extract of *Asparagus setaceus* leaves in the morning and afternoon.

2 MATERIAL AND METHODS

2.1 STUDY SITE

The experiments were carried out at the Natural Products Research Laboratory-LPPN of the Regional University of Cariri-URCA and at the Chemistry Laboratory of the Federal Institute of Education, Science and Technology of Ceará-IFCE, Juazeiro do Norte Campus.

2.2 OBTAINMENT OF BOTANICAL MATERIAL

The species was commercially acquired by the researcher at an open-air market in the city of Juazeiro do Norte-Ce and later cultivated in a private ornamental garden. To obtain the extract, fresh leaves of *Asparagus setaceus* were collected from this ornamental garden, Juazeiro do Norte-CE, located at 7°15'18.4"S 39°19'05.2"W in the morning (8 A.M) and in the afternoon period (2 P.M).

2.3 EXTRACT PREPARATION

The leaves of *Asparagus setaceus* (Kunth) were selected, dried at room temperature, and then crushed to increase the contact surface, submerged in P.A ethanol for cold extraction for a period of 72 hours, according to Simões et al. (2010). The mixture was subjected to filtration to remove impurities, and the distillation of the solvent took place on a rotary evaporator under reduced pressure at a controlled temperature between 30-60 °C. Extract yields were calculated based on the dry weight of the extract in the morning (50g) and afternoon (50g) compared to the weight of the leaves before extraction. The yield was 9.16% for the morning ethanol extract and 9.6% for the afternoon extract.



2.4 CHEMICAL PROSPECTING

Chemical prospecting was carried out according to the methodology of Matos (2009) and Simões et al. (2010) with the aim of elucidating classes of secondary metabolites such as flavonoids, alkaloids, tannins, among others. This assay is based on visual observation, color enhancement or precipitate formation after addition of specific reagents to sample solutions.

2.5 ALELOPATHIC ACTIVITY

2.5.1 Preparation Of Extract Concentrations

The test solution was obtained from 100 mg of ethanolic extract of *Asparagus setaceus* (Kunth) leaves, dissolved in 100 mL of 66% ethanol. The mixture was homogenized and transferred to a volumetric flask to obtain the Ethanol Extract at a concentration of 100% (Mother solution), from which concentrations of 50%, 25%, 12.5% and 6.25% were diluted (Treatments). Two control groups were used: distilled water and 66% ethanol alcohol (Andrade et al., 2017).

2.5.2 Bioassays For Allelopathic Potential Study

The bioassays of the ethanolic extracts had fractions that were in concentrations of 100, 50, 25, 12.5 and 6.25%, as previously described. Each treatment and controls consisted of five replications with 20 seeds of *Lactuca sativa* L. (lettuce). This receptor plant is used as a target of allelopathic studies due to its sensitivity, as even in lower concentrations of allelochemicals, its germination process can be compromised. Its germination is also fast (approximately 24 h), has linear growth, and is insensitive to differences in pH of the solutions (Rice, 1984; Souza, et al., 2005).

The experiments were carried out in Petri dishes with two filter paper discs moistened with 3 mL of the extract at different concentrations. The control was moistened in 3 mL of distilled water and 3 mL of 66% Ethanol Alcohol. The experiments were carried out in a seed germination chamber with biological oxygen demand (BOD) at a temperature of 25 °C and a photoperiod of 12 hours for seven days. The plates were left open for 12 hours to completely evaporate the solvent for bioassays with ethanol extract and concentrations (Mazzafera, 2003).



The pH of extracts at different concentrations was analyzed using a pH meter. Solutions with a pH greater than 6.0, a range considered optimal for germination, were adjusted using 0.1 N KOH and 1% HCl solutions (Macias, Gallindo; Molinillo, 2000).

2.5.3 Evaluated Parameters

The following parameters were evaluated: germination percentage (GP), germination speed index (GSI; every 24 hours) and stem and radicle length (assessed after seven days of sowing). Five seedlings of each replicate were used to measure the length of *L. sativa* stems and rootlets, totaling 25 seedlings per treatment and controls (Andrade et al., 2017).

2.6 STATISTICAL ANALYSIS

The results were evaluated using the linear regression model by multiple comparison. Two-way ANOVA followed by the Bonferroni or Tukey test using GraphPad Prism 6.0 software was used. Results at $p < 0.01$ will be considered statistically significant.

3 RESULTS E DISCUSSION

As shown in Table 1, the pH and mEEAS values ranged from 9 to 12, with the aEEAS showing pH of 8 to 12. These values were adjusted to the range of 6 to avoid interference with seedling growth and development (Table 1).

Table 1

pH values for morning and afternoon Ethanolic Extract of Asparagus setaceus (EEAS) leaves

TREATMENTS	mEEAS		aEEAS	
	Normal pH	adjusted pH	Normal pH	adjusted pH
CONTROL (ETHANOL 66%)	6,5	6	6,5	6
CONTROL (WATER)	5,5	6	5,5	6
6,25% EXTRACT	9	6	8	6
12,5% EXTRACT	12	6	10	5,8
25% EXTRACT	11	5,9	11	5,9
50% EXTRACT	12	6,1	11	6,1



100% EXTRACT	11	6	12	6
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Source: Authors (2023)

Sousa et al. (2020) describes that pH values 5.37-6.81 do not interfere with seedling germination and, therefore, are suitable for the development of most species, emphasizing that the pH between 5-7.0 of invasive species extracts are ideal for lettuce germination and development.

When under atypical conditions, pH values can negatively affect the growth and development of seedlings. Both acidity and alkalinity need to be adjusted to pH 6.0, as this range is suitable for germination. Fioresi et al. (2021) report that the interference of extracts containing sugars, amino acids and organic acids can mask the effect of secondary metabolites, when they are in extreme conditions.

Phytochemical analysis of the ethanol extract allowed qualification of 15 chemical constituents observed through the color change and formation of the precipitate. The chemical composition of mEEAS presented a greater amount when compared to the same extract prepared in the afternoon, as shown in Table 2.

Table 2

Chemical profile for morning and afternoon Ethanolic Extract of Asparagus setaceus (EEAS) leaves

CLASS OF SECONDARY METABOLITES															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
mEEAS	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-
aEEAS	-	-	+	-	-	+	+	+	+	+	-	+	+	+	-

1: Phenols; 2: Hydrolyzable tannins; 3: Condensed tannins; 4: Anthocyanins.

5: Anthocyanidins; 6: Flavones; 7: Flavonols; 8: Xanthones; 9: Chalcones; 10: Aurones; 11: Flavonols; 12: Leucoanthocyanidins; 13: Catechins; 14: Flavonones; 15: Alkaloids. (+) present; (-) absent

Source: Authors (2023)

According to Almeida (2017), the chemical composition of the same plant species, found in different locations, may vary according to soil type, temperature, climate, and altitude. A very relevant aspect is the time of collection, since this factor can influence the number of secondary metabolites. Some field studies have shown that time dependence also regulates a higher yield of plant compost, considering climatic influences, plant physiology and environmental conditions.

In the studies by Castro (2020), in addition to these factors described above, secondary metabolism can suffer interference from biotic and abiotic factors. The main factors, such as: atmospheric pollution, seasonality, development, circadian rhythm, and water availability;



ultraviolet radiation; nutrients and induction by mechanical stimuli or pathogen attack that can modify their quantity and quality. Therefore, when studying plant metabolism, variations that can coordinate, alter, and prevent the production of these compounds must be considered.

As for the difference between the ethanolic extracts produced in the morning and in the afternoon; according to the study by Puhl (2019), who evaluated a crude aqueous and ethanolic extract of the species under study, some plant compounds reach their maximum concentration during the hottest time of the year and close to noon, as in this study in the morning. However, at the end of the afternoon they reach a lower concentration at their peak of metabolites, and it is still not possible to say that all plants present this pattern.

Gobbo-Neto; Lopes (2006) describes medicinal plants and the factors that influence the content of secondary metabolites. Younger tissues generally have a higher biosynthetic rate of metabolites, such as: sesquiterpene lactones, phenolic acids, alkaloids, flavonoids, and stilbenes, when compared in stages of vegetative development, flowering, and senescence. There is a negative correlation between the production of allelochemicals and high metabolic activity, described in plants with periods of rapid tissue growth, as a decrease in the production of secondary metabolites (mainly phenolic derivatives). *Asparagus setaceus* shows slow and progressive growth in the morning (Pindel, 2017).

The phytochemical prospection brought great relevance to the research to previously know the chemical constitution of the species under study. As it is a qualitative analysis that shows little sensitivity to compounds at lower concentrations, in some situations these can be masked. Therefore, it is not safe to say that the compounds not detected in this analysis are not present in the species, they are just not found in the majority (Silva et al., 2021).

According to Magalhaes (2019), the *Asparagaceae* family has biological properties strictly related to the richness of phenolic compounds, including saponins, flavonoids, phenolic acids, and tannins, proving that they are potentially capable of performing the following effects: antimicrobial, antidiabetic, antioxidant, and diuretic. The saponins are the group that are found in large amounts and widely studied by the authors.

Lopez-Romero et al. (2017), when studying a plant of the same family, elucidated the ability of phenolic compounds, terpenes and saponins to alter membrane properties, resulting in changes in the hydrophobicity potential. Changes in the surface charge and in the integral conformation of the membrane have been described as a factor responsible for the extravasation of intracellular constituents and the apoptosis of plant membranes.

The genomic assays by Li et al. (2019), demonstrated a phylogenetic analysis that indicates that *A. setaceus* is closely related to *Asparagus officinalis* and *Asparagus*



schoberioides, when considering the genes of chemical composition. This genetic information is important to investigate the mechanisms of agricultural diseases and to characterize the resistance, genetics, and evolution of the species under study, corroborates Li et al. (2020).

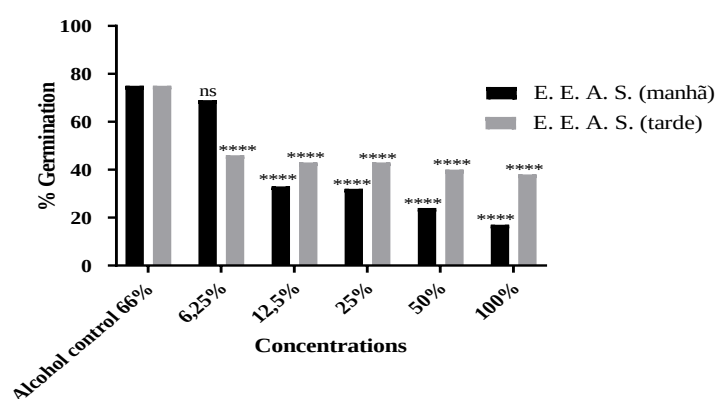
Schäfer et al. (2015), when qualifying changes related to the derivative storage of a species of the *Asparagus* genus, found that after storage the asparagus showed changes in the significant increase of phenolic monomers, cross-links of the phenolic cell wall and the polysaccharide profile of the plant was altered. In addition, the study showed structural changes in the post-harvest plant due to temperature interference, potentially reducing its medicinal capacity, reinforcing the change in the chemical profile shown in Table 1 of this work.

Secondary metabolites when released into the environment can interact with biological processes positively or negatively through direct or indirect pathways, according to Rockenbach et al. (2018), a synonym for allelochemical. This allelopathic interaction of plant extracts was initially described as a negative condition, but in this area, there are interactions capable of potentiating germination and plant development reactions, as well as clarifying the attraction of pollinators, interaction with herbivores, insects, and animals.

The bioassays performed with the ethanolic extract of *Asparagus setaceus* leaves showed statistical differences when observed in relation to the water and 66% alcohol control. Figure 1 demonstrates that there was a significant reduction in the germination of *Lactuca sativa* when compared to the Alcohol 66% control and only the Ethanol Extract in the morning at a concentration of 6.25% did not obtain a statistically significant result.

Figure 1

Graph of the germination percentage of *Lactuca sativa* exposed to different concentrations of the ethanolic extract of *Asparagus setaceus* leaves (EEAS) in the morning and afternoon. Values were expressed as S.E.M. means $\pm$ (n=5). "a" vs "control" Alcohol 66% when * = $p < 0,01$, ** = $p < 0,001$ e **** = $p < 0,0001$.

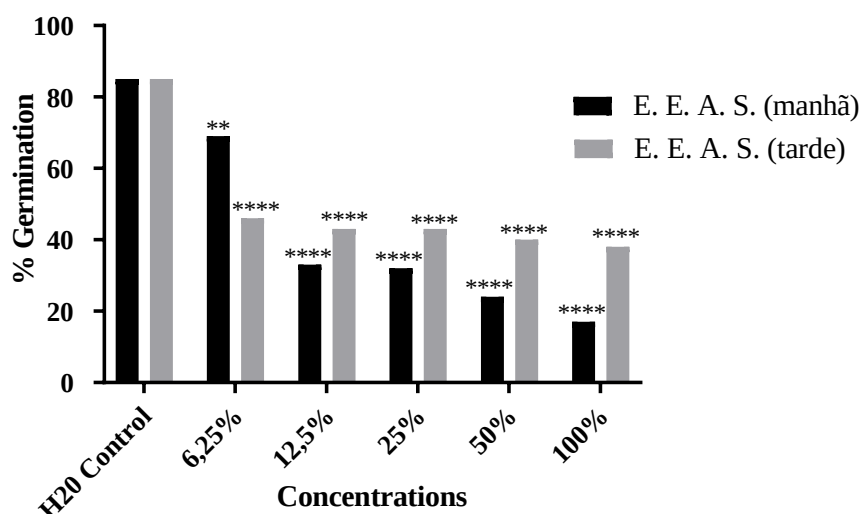




When evaluated against the water control (Figure 2) all concentrations presented a degree of significance that varies between $**p < 0.001$ and $****p < 0.0001$. The mEEAS at the lowest concentration showed a different reduction in the germination of *Lactuca sativa* when compared to the others. However, the afternoon extract remained with a higher degree of significance, thus inhibiting the percentage of germination at all concentrations.

Figure 2

Graph of the germination percentage of *Lactuca sativa* exposed to different concentrations of the ethanolic extract of *Asparagus setaceus* leaves (EEAS) in the morning and afternoon. Values were expressed as S.E.M. means $\pm$. ($n=5$). "a" vs "control" H_2O when $* = p < 0.01$, $** = p < 0.001$ and $**** = p < 0.0001$.



According to Silva and Pinto (2017), the development of seedlings becomes more sensitive to allelochemicals than the speed of germination, since their metabolism is directly affected by these substances, which should act on the binding of the receptor plants' membranes by or penetrating directly into the cell. This explains why germination showed a reduction in almost all concentrations of the morning and afternoon extract.

Formigheiri et al. (2018), showed that phenolic compounds such as: coumaric acid, catechin, phenols, tannins, among others, can change the physiology of plants by several mechanisms. By interfering with early root wall lignification, they alter root morphology and decrease cell division. These interferents may have influenced the reduction of *Lactuca sativa* germination, especially in the mEEAS since some of these compounds are present only in this extract (Table 1).



As demonstrated by Pereira et al. (2018), depending on the concentration of metabolites released by the plant into the environment, these can limit a germination pattern that will interfere with the plants around it. Alkaloids, coumarins, phenols, flavonoids, anthraquinone heterosides, tannins and terpenes are classes that present toxicity on cells justified by the alteration of the permeability of plant membranes and defects in the cytoskeleton. These findings justify the allelopathic activity as they negatively alter the germination and development of seedlings and may even lead to death.

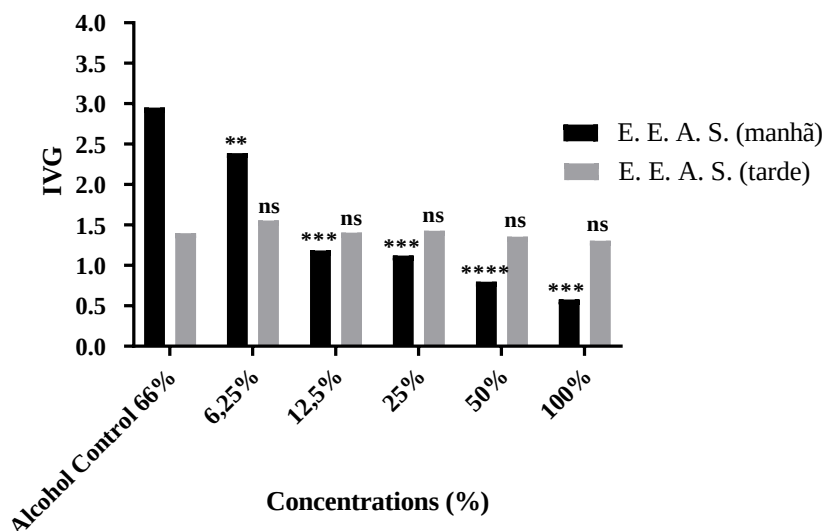
There are numerous factors that can influence seedling germination. The original location where the plant grew would be one of them since the environment where it is inserted can condition or conceal the presence of secondary metabolites. As a result, the period or place may provide different results regarding the presence or intensity of these compounds. According to Moreira et al. (2021), seasonality can intervene in the diversity and production of phytoconstituents, thus justifying the different results presented in Figures 1 and 2.

The findings of this study show that the ethanol extract has a greater ability to interfere with germination than the other extracts that use other types of solvents. Silva et al. (2021) found that the germination parameters of *Lactuca sativa* were not influenced by the application of the aqueous extract, but when the ethanolic alcohol-based extract was used, there were adverse effects on germination. It is a fact that the physicochemical properties of the solvent are responsible for the number of compounds extracted from the material, especially phenolic compounds (a class that has allelopathic potential) which are more easily extracted in organic solvents that are less polar than water.

The allelopathic effect can manifest itself directly through germination, influence on the speed of germination or other characteristic of the germination process. Figures 3 and 4 are the Germination Speed Index (GSI) against the 66% ethanol and H₂O control.

**Figure 3**

Graph of the germination speed index (I VG) of *Lactuca sativa* exposed to different concentrations of the ethanolic extract of *Asparagus setaceus* leaves (EEAS) in the morning and afternoon. Values were expressed as S.E.M. means $\pm$. (n=5). "a" vs "control" alcohol 66% when * = $p < 0.01$, ** = $p < 0.001$ and **** = $p < 0.0001$.

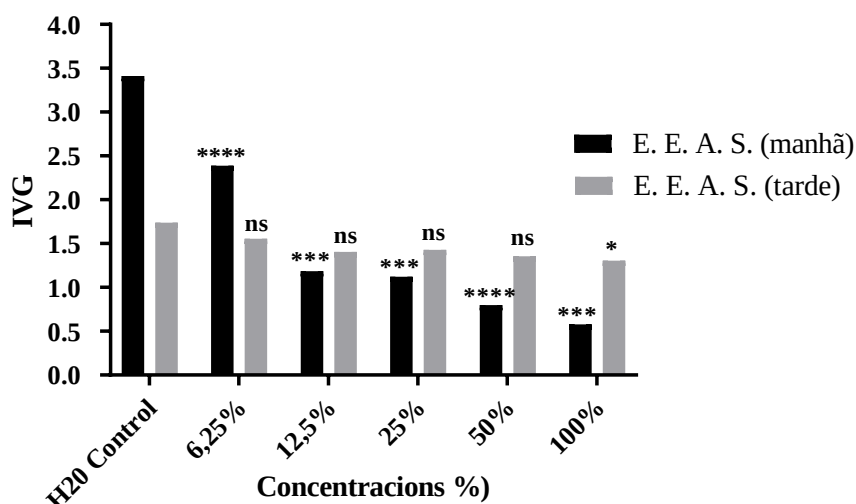


The mEEAS GSI showed a reduction from the test's 6.25% concentration, thus maintaining a constant fall of this parameter as the concentration increased. The plateau of 50% reduction in the germination speed index was obtained at a concentration of 12.5%, with a significant **** $p < 0.0001$. Using the ethanol 66% alcohol control as a comparison, the aEEAS did not present significant results in any of the concentrations.

In Figure 4, mEEAS caused a delay in the germination of *Lactuca sativa* at all concentrations tested when compared to the H₂O control. The 50% reduction in germination varied between concentrations of 6.25% - 12.5% for the extract produced in the morning. However, the afternoon ethanol extract showed four concentrations without significant results and only concentration of 100% (highest concentration) with a significance of * $p < 0.01$.

**Figure 4**

Graph of the germination speed index (IVG) of *Lactuca sativa* exposed to different concentrations of the ethanolic extract of *Asparagus setaceus* leaves (EEAS) in the morning and afternoon. Values were expressed as S.E.M. means $\pm$. ($n=5$). "a" vs "control" H₂O when * = $p < 0.01$, ** = $p < 0.001$ and **** = $p < 0.0001$.



Germination time is a very important factor in the allelopathic process, as it has a direct effect on seedling growth and development. Rocha et al. (2021), cite that seeds that germinate more slowly can give rise to seedlings that have reduced size, making them more susceptible to stressors and predation itself. Thus, these have a lower chance of survival when competing for resources.

Srivastava; Shukla; Kalunke (2018), in studies on *Asparagus racemosus*, identified a significant number of triterpene and steroidal saponins in roots, leaves and fruits, the leaves particularly also containing flavonoids. From this discovery it was possible to identify which species of the genus encode enzymes involved in the biosynthesis or maintenance of chemical compounds (allelochemicals) for the regulatory mechanism and plant metabolism, thus influencing the germination speed.

The mEEAS significantly reduced germination speed. This data was also observed by Silva et al. (2021), who, when evaluating the ethanol extract against *Lactuca sativa* seedlings, observed a decrease in germination speed, justifying this finding by the high concentrations of phenolic compounds capable of altering the osmotic pressure, inhibiting the process of elongation and division of the roots, and regulating cell membrane hyperpolarization through the ATP pump, directly interfering with seedling development and growth.



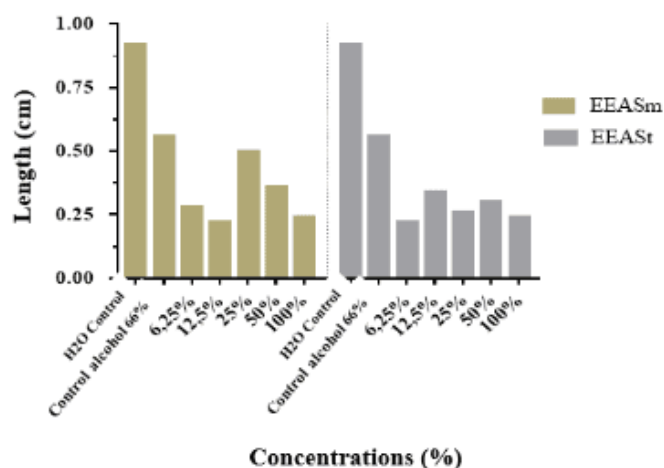
aEEAS showed an important reduction in germination only at the highest concentration when compared to the H₂O control (Figure 4). Phenolic compounds play an important role in plant defense and survival, in small amounts they can promote cell growth, are considered natural antioxidants and, therefore, prevent protein and transporters denaturation, thus optimizing plant enzymatic activity, cites Fiorezi et al. (2021). These factors may be involved with the result presented for this extract, since it does not have detectable levels of phenolic compounds by the method used (Table 1).

Upadhyay et al. (2019), verified whether a species of the *Asparagus* genus had the ability to promote germination and tolerance to abiotic stress of a transgenic plant. In their study, it was found that the species encodes a protein that accelerates the early germination of transgenic seeds and that it has the ability to generate drug-resistant seeds. This activity was attributed due to the amount of steroidal saponins that the species has.

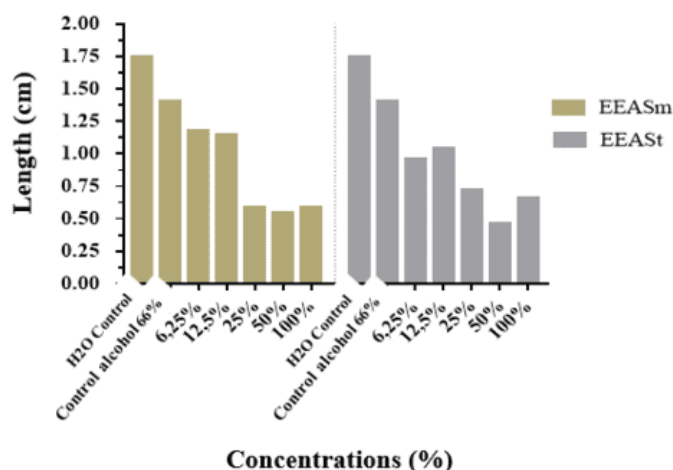
Macías; Mejías; Molinillo (2019), denote that the compounds will only effect the allelopathic activity if they are released/accumulated in sufficient quantities. However, the process is complex, the plant suffers environmental interference from ultraviolet and infrared radiation and degradation by the Microorganisms themselves. In some cases, the structures of allelochemicals can suffer or cause oxidative stress leading to degradation of the receptor species, in other situations the metabolites lead to greater growth of the target plant even under adverse conditions. For these reasons, the heterogeneity of results in allelopathic trials should be considered.

**Figure 5**

Graph of the average stem length of seedlings of *Lactuca sativa* exposed to different concentrations of the ethanolic extract of *Asparagus setaceus* leaves (EEAS) in the morning and afternoon. Values were expressed as S.E.M. means $\pm$. (n=5). "a" vs "control" H₂O and alcohol 66% when * = $p < 0.01$, ** = $p < 0.001$ and **** = $p < 0.0001$.

**Figure 6**

Graph of the average root length of seedlings of *Lactuca sativa* exposed to different concentrations of the ethanolic extract of *Asparagus setaceus* leaves (EEAS) in the morning and afternoon. Values were expressed as S.E.M. means $\pm$. (n=5). "a" vs "control" H₂O when * = $p < 0.01$, ** = $p < 0.001$ and **** = $p < 0.0001$.



Evaluating both controls, the aEEAS significantly inhibited stem growth of the seedlings tested, although the results presented variations, all were significant and obtained a **** $p < 0.0001$. In root length, both extracts showed inhibition, which was more evident as concentrations increased, as seen in Figure 6.



For Silva et al. (2021), allelochemicals have an enormous capacity to regulate root growth, as their interaction with phytohormones takes place through phenolic compounds that will act on hormones such as auxins, a compound responsible for root growth. This behavior can be attributed mostly to the morning extract since it presented phenolic compounds and a greater reduction of the radícula compared to the afternoon extract.

Some asparagus are self-toxic, according to Noguchi et al. (2017). Phenolic compounds have been described with this potential, and it indicates that some are released into the soil by root exudation. The accumulation of these substances can inhibit the growth of other plants and reduce the productivity of the *Asparagus* genus, which is called asparagus decline. This factor may explain the reduction in root and stem growth in this test.

There are several substances responsible for inhibiting the growth of seedlings. Noguchi; Nakamura; Okuda (2018), when evaluating the quality of the harvest of *Asparagus officinalis* L., the autotoxic effect of trans-cinnamic acid inhibited the growth of seedlings at concentrations greater than 10 μ M. Therefore, this polyphenolic may be responsible for the autotoxic effect of asparagus soils and may be partly responsible for the "asparagus decline" and the "asparagus replanting problem", as shown in Figure 5 and 6.

The differences between the morning and afternoon extract averages can be explained by Noperi-Mosquedaa et al. (2019), who, when testing with allelopathy against *Lactuca sativa*, observed that the leaves and rhizomes of a species of the *Asparagaceae* family presented different phenolic compounds depending on the collection period, and that these reduced the germination of the recipient plant and the growth of the epicotyl, an important structure for the initial stage of an embryonic plant. The results are useful for a better understanding of how phytotoxicity works.

The ethanolic extract of the leaves is presented as a basis for an in-depth study of the inhibitory potential of germination, since it can concentrate potentially herbicidal substances in its composition, due to its polar and non-polar ends, thus diluting polar and non-polar substances, such as allelochemicals. However, Puhl (2019) concluded that tests using the ethanol extract should consider variables found in the field, such as the period of collection of plant material.

4 CONCLUSIONS

The ethanolic extract of *Asparagus setaceus* leaves morning and afternoon showed secondary metabolites in common, such as: flavonoids, saponins, anthocyanidins and phenolic



compounds. The negative interference observed in the GSI and in the development of seedlings occurred more significantly in the mEEAS, due to having more significant concentrations of metabolites different from the extract produced in the afternoon, justifying the better allelopathic activity. The combined action of these compounds and the biotic and abiotic factors, such as: humidity, luminosity, temperature, and soil pattern must be considered when evaluating the different results found. However, more studies are needed to identify and even isolate them to investigate the specific constituents that caused the allelopathic effects verified in this research.

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