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INVESTIGATION OF THE BIOHERBICIDAL POTENTIAL OF ROSEMARY ON LETTUCE GERMINATION AND INITIAL DEVELOPMENT

INVESTIGAÇÃO DO POTENCIAL BIOHERBICIDA DE ALECRIM SOBRE A GERMINAÇÃO E O DESENVOLVIMENTO INICIAL DE ALFACE

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ABSTRACT

The objective of this study was to evaluate the bioherbicidal potential of rosemary extract (*Salvia rosmarinus* Spenn.) through a bioassay conducted on the germination and initial development of lettuce (*Lactuca sativa* L.). For this purpose, the experiment was conducted with five treatments: distilled water (negative control), 1.5% glyphosate (positive control), ethanol (negative control), 100% rosemary hydrolate, and 10% rosemary essential oil. Each treatment was performed with four replicates in a completely randomized design, where each replicate consisted of 50 lettuce seeds on germination paper in Petri dishes containing 3.4 mL of the respective treatment. The Petri dishes were placed in a B.O.D. germination chamber regulated at a constant temperature of 20 °C and a photoperiod of 12 hours for seven days. During this period, daily counts of germinated seeds were performed, and at the end, the radicle and aerial part of the lettuce seedlings were measured. This yielded the germination percentage (%G), germination speed index (GSI), radicle length (CR), and aerial part length (CPA). The data were submitted to Analysis of Variance (ANOVA) and the Scott-Knott test of means ($p<0.05$). The results indicated that rosemary extracts caused a significant reduction in germination and initial development of lettuce. The hydrolate at a concentration of 100% showed inhibition in all variables analyzed, indicating bioherbicidal potential.

Keywords: Allelopathy; Hydrosol; Secondary metabolites; *Rosmarinus officinalis* L.; *Salvia rosmarinus* Spenn.

RESUMO

O objetivo deste trabalho foi avaliar o potencial bioherbicida do extrato de alecrim (Salvia rosmarinus Spenn.) através do bioensaio realizado sobre a germinação e desenvolvimento inicial de alface (Lactuca sativa L.). Para isso, o experimento foi conduzido com cinco tratamentos: água destilada (controle negativo), glifosato 1,5% (controle positivo), etanol (controle negativo), hidrolato de alecrim 100% e óleo essencial de alecrim 10%. Cada tratamento foi realizado com quatro repetições em Delineamento Inteiramente Casualizado, onde cada repetição foi composta de 50 sementes de alface sobre papel germitest em placas de Petri contendo 3,4 mL do repectivo tratamento. As placas de Petri foram dispostas em câmara de germinação B.O.D. regulada a uma temperatura constante de 20 ºC e fotoperíodo de 12 horas durante sete dias. Nesse período foram realizadas contagens diárias das sementes germinadas e, ao final, foi medida a radícula e parte aérea das plântulas de alface. Com isso, obteve-se a porcentagem de germinação (%G), índice de velocidade de germinação (IVG), comprimento de radícula (CR) e comprimento de parte aérea (CPA). Os dados foram submetidos à

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Análise de Variância (ANOVA) e ao teste de médias Scott-Knott (p<0,05). Os resultados expuseram que os extratos de alecrim provocaram redução significativa na germinação e no desenvolvimento inicial de alface. O hidrolato na concentração de 100% demonstrou inibição em todas as variáveis analisadas, indicando potencial bioherbicida.

Palavras-chave: *Alelopatia; Hidrolato; Metabólitos secundários; Rosmarinus officinalis L.; Salvia rosmarinus Spenn.*

1 INTRODUCTION

Weeds exhibit aggressiveness toward cultivated crops due to their natural adaptability and the development of competitive abilities (Silva *et al*., 2021). These phenomena are associated with economic losses in the agricultural sector, as they result in diminished final crop yields, increased production costs, and indirect consequences for environmental and human health. The excessive use of chemical herbicides, such as glyphosate, has been demonstrated to promote the emergence of resistant biotypes, necessitating higher doses and more frequent applications of the product (Foles *et al*., 2023; Brito *et al*., 2018). Moreover, glyphosate has been classified as probably carcinogenic to humans (IARC, 2017).

The quest for natural substitutes for the extensive utilization of chemical herbicides has prompted research in the domains of natural and technological sciences, particularly those that concentrate on the application of bioactive compounds of microbial and plant origin (Silva *et al*., 2020; Chamoun, 2019; Fernandes *et al*., 2023; Yogeandra *et al*., 2025). Various methodologies are employed to obtain plant extracts, including infusion, maceration, and hydrodistillation. The latter, employed for the extraction of essential oils, generates hydrosol as a by-product, which is frequently discarded by industry. Despite its underexploration, this by-product demonstrates potential for application in various fields, including agriculture through sustainable and ecological practices (Almeida *et al*., 2024; Lima *et al*., 2024).

Rosemary hydrosol is regarded as a prospective reservoir of phenolic compounds and has previously exhibited elevated antioxidant levels in comparison to the aqueous extract of the non-distilled plant (Wollinger *et al*., 2016). The identified compounds include rosmarinic acid, carnosol, carnosic acid, flavonoids, and derivatives of caffeic acid (Celano *et al*., 2017). Despite their low water solubility, the high temperature of hydrodistillation promotes their dissolution (Rafya *et al*., 2024).

Considering the aforementioned points, research endeavors investigating the utilization of essential oils and hydrosols in bioassays with bioindicator species, such as *Lactuca sativa* L. (Brazil, 2009), hold significant pertinence to the advancement of sustainable technologies aimed at the ecological management of weeds. Consequently, plant species such as *Salvia rosmarinus* Spenn., which are extensively employed in traditional medicine due to their antioxidant, anti-inflammatory, antibacterial, and antimicrobial properties (Chaves, 2022; Porte & Godoy, 2001), have demonstrated potential for bioherbicidal investigation due to their recognized bioactivity and antiproliferative effect, specifically the inhibition of cell division (Frescura, 2014; Chamoun, 2019).

Accordingly, the present study aimed to evaluate the bioherbicidal potential of rosemary (*Salvia rosmarinus* Spenn.) through the application of its extracts (essential oil and hydrosol) on the germination and early development of lettuce (*Lactuca sativa* L.).

2 MATERIAL AND METHODS

The bioassay was conducted in a laboratory at the Department of Biology, Center for Natural and Exact Sciences (CCNE), Federal University of Santa Maria (UFSM), Santa Maria campus, RS, Brazil. To analyze the bioherbicidal potential of rosemary (*Salvia rosmarinus* Spenn.) on the germination and early development of lettuce (*Lactuca sativa* L.), rosemary hydrosol and essential oil were provided by the company Vimontti - Agroindústria São Caetano Ltda®, while seeds of American lettuce cv. G.L. 659, free of agrochemicals, was obtained from a local supplier.

Rosemary hydrosol was obtained by hydrodistillation of the fresh aerial parts of the organically grown plant, presenting a clear appearance and characteristic odor. Both the essential oil and hydrosol were stored at 4°C under refrigeration in amber bottles until use.

In accordance with the experimental design, 50 lettuce seeds were meticulously placed on a double sheet of Germitest paper in Petri dishes; with each dish containing 3.4 mL of the proposed treatment (see Table 1 for details). The experimental design was executed in accordance with a completely randomized design (CRD), comprising five distinct treatments, each with four replicates. It is noteworthy that each Petri dish was regarded as a single replicate in the statistical analysis.

Table 1 - Treatments used to evaluate the allelopathic effect of rosemary (*Salvia rosmarinus* Spenn.) on the germination and early development of lettuce (*Lactuca sativa* L.).

Treatment	Description
W	Distilled water (Negative control)
G	Glyphosate 1.5% (Positive control)
E	Absolute ethanol (Negative control)
HD	100% Rosemary Hydrosol
OE	10% Rosemary essential oil

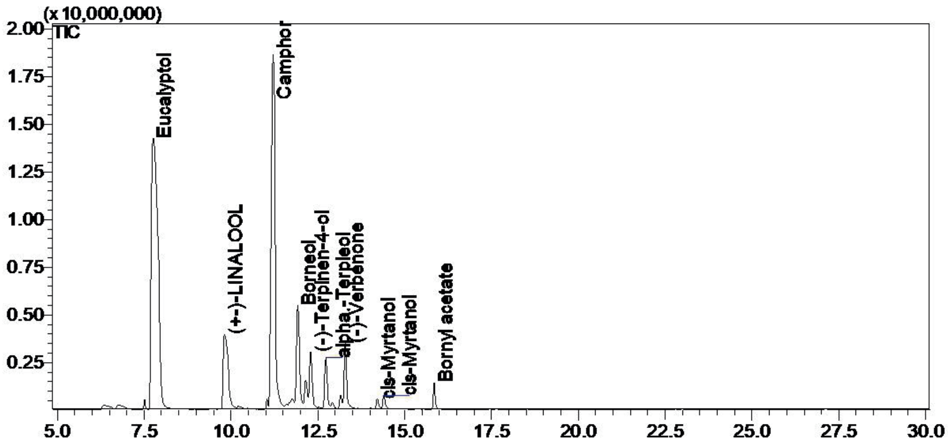
Source: Authors.

For the treatment of HD, the hydrosol was utilized in its pure form, comprising major compounds such as eucalyptol (42.84%), camphor (34.24%), and borneol (7.91%), along with verbenone (4.45%), terpinen-4-ol (4.14%), α-terpineol (3.29%), bornyl acetate (1.30%), and cis-myrtanol (1.13%) (Figure 1). The identification of compounds present in both the hydrosol and the essential oil was carried out by the supplying agro-industry employing gas chromatography-mass spectrometry (GC-MS).

The rosemary essential oil treatment (OE) was prepared by dilution in absolute ethanol to a concentration of 10%. Chromatographic analysis (GC) revealed the following compounds: α-pinene (38.8%), eucalyptol (21.86%), camphene (5.55%), limonene (4.5%), verbenone (3.75%), α-terpineol (1.74%), β-pinene (2.41%), bornyl acetate (2.56%), β-caryophyllene (0.81%), and other components in concentrations below 2% (Figure 2). In addition to these treatments, distilled water (W) and absolute ethanol (E) were utilized as negative controls, and glyphosate 1.5% (G) was employed as the positive control.

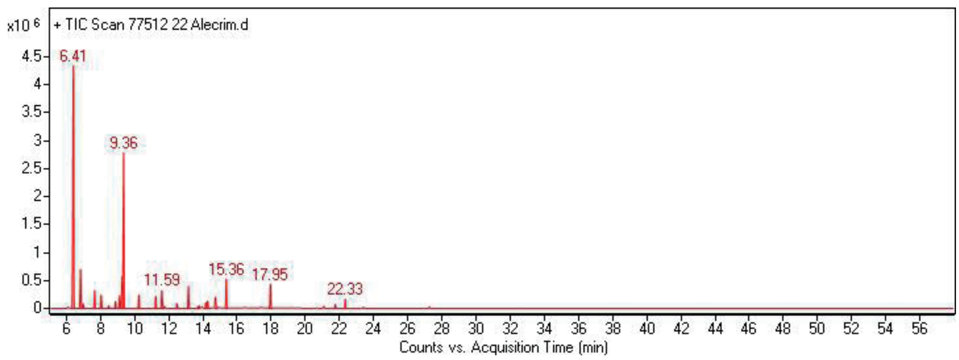
The Petri dishes containing the lettuce seeds and their respective treatments were placed in a B.O.D. germination chamber that was set to a 12-hour photoperiod and a constant temperature of 20°C. The germination process was meticulously monitored over a period of seven days, and seeds that exhibited a radicle length of 2 mm or greater were designated as germinated. On the seventh day, the plates were removed from the germination chamber for a final count, during which the shoot and root system were measured with a digital caliper.

Figure 1 - Chromatogram of the rosemary (*Salvia rosmarinus*) hydrosol sample used.



Source: Central Analítica (UNISC)

Figure 2 - Chromatogram of the rosemary (*Salvia rosmarinus*) essential oil sample used.



Source: Central Analítica (UNISC)

Based on these procedures, the following parameters were obtained: germination percentage (%G), germination speed index (GSI), radicle length (RL), and shoot length (SL). Germination percentage (%G) was calculated using Formula 1, where G = total number of germinated seeds; S = total number of seeds sown. For the calculation of the germination speed index (GSI), Formula 2 was used, where Gi = number of seeds germinated on the i-th day of counting, and *Ni* = number of days from sowing to the i-th day (*i*.e., *Ni* = *i*).

$$\%\,G = \left(\frac{G}{S}\right)\,x\,100 \quad (1)$$

$$GSI = \sum_{i=1}^{n} \left(\frac{Gi}{Ni}\right) \quad (2)$$

The data obtained were subjected to analysis of variance (ANOVA), and means were compared using the Scott-Knott test at a 5% significance level ($p < 0.05$).

3 RESULTS AND DISCUSSION

The analysis of variance (ANOVA) was significant for both germination percentage (%G) and germination speed index (GSI) of *Lactuca sativa* seeds under the different treatments (Table 2).

Table 2 - Results of the analysis of variance (ANOVA) for germination percentage (%G) and germination speed index (GSI) of lettuce (*Lactuca sativa* L.) seeds subjected to different treatments.

Treatment	Description	G (%)	GSI
W	Distilled water (Negative control)	99.0 a	94.39 a
G	Glyphosate 1.5% (Positive control)	90.5 b	57.82 b
E	Absolute ethanol (Negative control)	0.0 c	0.00 c
HD	100% Rosemary Hydrosol	0.5 c	0.04 c
OE	10% Rosemary essential oil	0.0 c	0.00 c

According to the Scott-Knott test ($p < 0.05$), means followed by the same letter do not differ statistically from each other. Source: Authors.

The negative control, which involved distilled water (W), exhibited 99% germination, thereby demonstrating the viability of the seeds. In comparison with the control, treatments G, E, HD, and OE exhibited a marked interference with germination, exhibiting a significant inhibition (Figure 3).

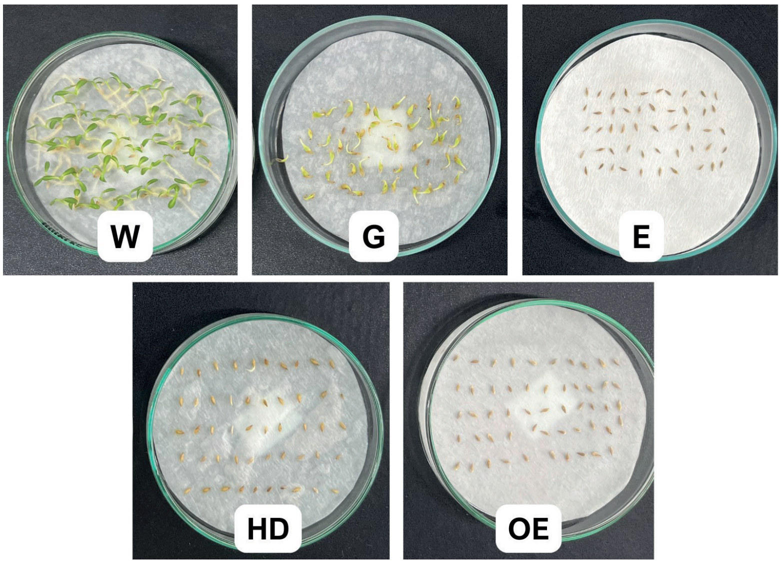
The treatment with 1.5% glyphosate (G), utilized as a positive control, exhibited 90.5% seed germination, exhibiting a significant difference from all other treatments. However, this inhibitory effect was lower than that observed with the rosemary (*Salvia rosmarinus*) extracts. The administration of a hydrosol (HD) at a concentration of 100% resulted in a germination rate of 0.5%, while the application of a 10% essential oil (EO) did not yield any germination. When subjected to a comparison using the Scott-Knott mean test ($p<0.05$), the HD and EO treatments did not demonstrate a statistically significant difference, suggesting that both treatments exerted comparable inhibitory effects.

These results suggest that the major compounds present in higher levels in the hydrosol (42.84% eucalyptol and 34.24% camphor) and in the essential oil (38.8% α-pinene and 21.86% eucalyptol) exerted phytotoxic activity on lettuce seed germination.

Frescura (2014) conducted a study to examine the impact of rosemary essential oil at concentrations of 3% and 10% on the cell cycle of *Allium cepa.* The findings revealed a heightened antiproliferative effect at the 10% concentration. In the present investigation, the complete inhibition of germination in the 10% essential oil (EO) treatment may be associated with the action of its compounds and with the volatilization of the essential oil and the ethanol used as solvent. The treatment with absolute ethanol (E) also exhibited no germination, suggesting that the compounds of the essential oil may not be the only ones responsible for the observed inhibitory effect. Consequently, it is advised that subsequent bioassays contemplate diverse methodologies for essential oil dilution or emulsification, thereby ensuring outcomes that are more definitive.

In a 2019 study, Chamoun examined the effects of rosemary nanoemulsions on lettuce seed germination. The study found a substantial reduction in seed germination at concentrations of 7 mg·mL^{-1} and 10 mg·mL^{-1}, suggesting the occurrence of allelopathic action by compounds such as eucalyptol (1,8-cineole) and α-pinene.

Figure 3 - Representative sampling of the effects of different treatments on lettuce (*Lactuca sativa* L.) seeds.



W: Distilled water (negative control); G: 1.5% glyphosate (positive control); E: Absolute ethanol (negative control); HD: 100% rosemary hydrosol; OE: 10% rosemary essential oil. Source: Authors.

A similar effect was observed when *Salvia officinalis* hydrosol (42% eucalyptol) was tested on lettuce, inhibiting its germination (Politi *et al.*, 2022), and when *Lavandula angustifolia* essential oil was evaluated for its bioherbicidal potential against *Echinochloa crus-galli*, demonstrating phytotoxic effects on germination and weed development, which the authors attributed to eucalyptol (1,8-cineole) (Ibáñez *et al.*, 2019).

The variable germination speed index (GSI) exhibited significant variation among the treatments, with those involving distilled water (A) and 1.5% glyphosate (G) demonstrating notably higher means compared to the other treatments, though these differences were not statistically significant. The treatments with ethanol (E), 100% rosemary hydrosol (HD), and 10% rosemary essential oil (EO) did not differ from one another, indicating that the rosemary extracts exerted a greater effect on germination speed, causing a significant reduction.

In comparison with the control group that received distilled water, the reduction in GSI observed in the rosemary treatments may suggest the presence of allelochemicals capable of interfering with the translocation of nutrients from the endosperm to the embryo, thereby inhibiting its development (Azambuja *et al*., 2010). According to Lanzoni *et al.* (2018), the reduction in GSI may be associated with decreased seed vigor. As is the case with *Salvia rosmarinus*, other species belonging to the Lamiaceae family have also been found to contain compounds such as eucalyptol (1,8-cineole) and α-pinene, which may be related to the reduction in GSI. In this regard, Neto *et al.* (2024) observed a significant reduction in GSI in lettuce seeds treated with *Hyptis suaveolens* (L.) Poit. hydrosol at concentrations of 50% and 100%.

In the analysis of early seedling development through the measurement of shoot length (SL), radicle length (RL), and total length (TL), significant differences were observed among treatments (Table 3). The rosemary extracts (HD and EO) exhibited the shortest lengths for all three variables, differing from the distilled water (A) treatment, and did not differ from each other. The administration of 100% hydrosol (HD) resulted in mean values of 0.95 millimeters for RL, 0.72 millimeters for SL, and 1.67 millimeters for TL. In the treatment with 10% essential oil (EO), no measurable seedlings were formed due to total germination inhibition.

Table 3 - Results of the analysis of variance (ANOVA) for radicle length (CR), shoot length (CPA) and total length (CT) of lettuce seedlings (*Lactuca sativa* L.) subjected to different treatments.

Treatment	Description	Rl	SL	LT
W	Distilled water (Negative control)	22.94 a	7.46 a	30.40 a
G	Glyphosate 1.5% (Positive control)	2.75 b	3.12 b	5.87 b
E	Absolute ethanol (Negative control)	0.00 c	0.00 c	0.00 c
HD	100% Rosemary Hydrosol	0.95 c	0.72 c	1.67 c
OE	10% Rosemary essential oil	0.00 c	0.00 c	0.00 c

According to the Scott-Knott test ($p<0.05$), the means followed by the same letter do not differ statistically from each other. The lengths are expressed in millimeters (mm). Source: Authors.

The observed efficacy of rosemary hydrosol is consistent with the findings of germination analyses (G% and GSI), suggesting that the components present in the extract are associated with biochemical properties that consistently interfere with the reduction of multiple variables related to lettuce development.

In the initial phases of lettuce development, Chamoun (2019) reported that rosemary (*Salvia rosmarinus*) essential oil nanoemulsions at concentrations of 5 $mg \cdot mL^{-1}$, 7 $mg \cdot mL^{-1}$, and 10 $mg \cdot mL^{-1}$

exhibited a more pronounced inhibitory effect on radicle growth compared to shoot development. However, Neto *et al.* (2024) observed a reduction in shoot development, with no significant effect on radicle length, in lettuce seedlings exposed to 50% and 100% concentrations of *Hyptis suaveolens* hydrosol. The author interpreted the greater investment in root development as a resistance mechanism of the seedling in response to chemical stress, since primary root development is essential for water and nutrient uptake.

These patterns underscore the intricacy of the mechanisms involved in responses to allelopathic compounds. Among the likely compounds responsible for inhibition, eucalyptol (1,8-cineole) is particularly noteworthy. According to Romagni *et al.* (2000), the compound in question was a potent inhibitor of germination, root and mitotic development in *Echinochloa crusgalli* (L.) p. Beauv and *Cassia obtusifolia* L.

In addition to the volatile compounds present in hydrosols and essential oils, phenolic compounds may also exert allelopathic effects. Puig *et al.* (2018) associated the inhibition of lettuce germination with these compounds, whereas eucalyptol was identified as one of the volatile constituents responsible for reducing seedling growth. Therefore, further studies should address the isolated effects of non-volatile compounds.

Identified in several essential oils, eucalyptol (1,8-cineole), is also a major constituent of *Nepeta flavida* essential oil (30,80%), which, tested on *Lepidium sativum*, *Raphanus sativus*, and *Eruca sativa* seeds, caused complete germination inhibition at concentrations of 4.0 and 8.0 µl/ml (Bozok, 2018). Given the abundance of the compound in the hydrosol and essential oil used in this study, its role in the inhibitory effect is reinforced.

In investigations on bioherbicidal potential, germination inhibition is the most frequently reported effect (Romagni *et al.*, 2000; Lanzoni *et al.*, 2018; Chamoun, 2019; Neto *et al.*, 2024), where eucalyptol and α-pinene being among the main constituents associated with phytotoxic activity. These compounds act by reducing seedling growth and vigor, while also impairing essential physiological processes, including cellular respiration, pigment synthesis, and cell membrane permeability (Dolianitis *et al.*, 2025; Radhakrishnan *et al.*, 2018).

Reinforcing the inhibitory effect of eucalyptol, studies have confirmed its antiproliferative activity against cancerous and microbial cells (Luca *et al.*, 2020; Abdalla *et al.*, 2020; Merghni *et al.*, 2023). Accordingly, the results of the present study indicate that this compound is one of the main contributors to the observed phytotoxic activity with potential bioherbicidal effects. Further studies involving the isolation of eucalyptol are also needed.

4 FINAL CONSIDERATIONS

In the context of the study, the rosemary (*Salvia rosmarinus* Spenn.) extracts were examined to ascertain their bioherbicidal potential. Among the extracts, the hydrosol exhibited bioherbicidal potential at the tested concentration, demonstrating efficacy in hindering the germination and early development of lettuce (*Lactuca sativa* L.). However, the rosemary essential oil's bioherbicidal potential remained unconfirmed due to the possibility of complete volatilization before exerting an effect on the lettuce seeds.

The biochemical compounds present in the rosemary extracts studied, such as eucalyptol (1,8-cineole), camphor, and α-pinene, have been shown to be associated with the bioherbicidal potential of different species. This supports the inhibition of germination and early development of the bioindicator species (*Lactuca sativa* L.) observed in this study.

The potential of rosemary hydrosol to impede lettuce germination and early development indicates a prospect for the utilization of essential oil industry remnants in the development of technologies focused on more sustainable agricultural management. This approach would transform what would otherwise be considered waste into a value-added resource.

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