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Allelopathic Influence of *Rosmarinus officinalis* (L) Aqueous Extracts on Barley Plants

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ABSTRACT

This is an investigation of Allelopathic Influence of Rosmarinus officinalis L. Aqueous Extracts on Barley Plants. All the samples are prepared from Libyan varieties of Rosmarinus officinalis specimens were gathered, air-dried, and ground, yielding a 20% aqueous extract. Dilutions (16%, 12%, 8%, 4%) and control (0% distilled water) were prepared. Dilution followed the equation C2×V2=C1×V1 After a week in the lab with two irrigations, root and stem lengths were measured. Statistical analyses, including one-way ANOVA and multiple comparisons, were conducted using SPSS 28 to interpret the extract's impact. The results of the experiment revealed a significant difference in Barley root measurements among the experimental conditions, as evidenced by a significant F-statistic (F(4, 10) = 15.58, p < .001). The Between Groups comparison, with a sum of squares of 38.85 and 4 degrees of freedom, resulted in a mean square of 9.71. Results also revealed a significant difference in barley stem measurement among the experimental conditions, as indicated by a highly significant F-statistic (F(4, 10) = 45.777, p < .001). These differences indicate that the presence of Rosmarinus officinal L.is Weed extract, under various concentrations and conditions, distinctly influenced (negatively) the growth parameters of barley plants.

Keywords: Rosmarinus officinalis L., allelopathic interactions, Aqueous Extracts, Barley Plants.

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1. Introduction

Allelopathy refers to the direct or indirect effect of plants upon neighboring plants or their associated micro flora or micro fauna by the production of allele chemicals that interfere with the growth of the plant (IAS, 2018). The effect can be either positive or negative on the growth of the surrounding plants. The word allelopathy is derived from two separate Greek words, allelon meaning of each other or mutual and pathos meaning to suffer or feeling. Even though the term 'allelopathie' was first used by Austrian scientist Hans Molisch in 1937 (Willis, 2007). Many plant species produce phytochemicals that enable them to inhibit or suppress the germination or growth of other plants (Baziar,et.al, 2014, Sitthinoi et.al, 2017). These bio chemicals are also called allele chemicals and generally constitute phenolic compounds, saponins, terpenes, steroids, alkaloids, and quinones (Dayan et al, 2009) Moreover, a number of allele chemicals from potentially allelopathic plants species have already been isolated and have proven to successfully inhibit weed germination and growth (Suksungworn etal, 2016) . The allele chemicals released from the plants act as a defense system against microbial attack, herbivore predation, or competition from other plants (Kong et al., 2019). The study of allelopathy is a sub-discipline of chemical ecology that focuses on the effects of chemicals produced by plants or microorganisms on the growth and development of other plants in natural or agricultural systems (Einhellig, 1995).

In the intricate arrangement of ecosystems, plants engage in silent warfare for resources, territory, and survival. Allelopathy, a phenomenon where plants release biochemical compounds to influence the growth and development of neighboring vegetation, is a testament to the fascinating) carnosic acid (warfare occurring beneath the soil and above the ground (Macías,et al. 2020). In this botanical, certain aromatic herbs and hardy shrubs such as Rosmarinus (rosemary), Thyme, Artemisia (wormwood), and Dryas have emerged as key players, wielding their allelopathic potential to shape the destiny of surrounding flora (Gurmani, et al. 2020). The research presented here will explore Allelopathic Influence of Rosmarinus officinalis Aqueous Extracts on Barley Plants. Rosmarinus is not merely culinary delights or ornamental additions to our gardens; they are botanical alchemists capable of producing a cocktail of allelochemicals that can profoundly impact the growth and vitality of neighboring plants. The allelopathic influence of these species on wheat and barley, two of the world's essential cereal crops, raises questions about the delicate balance between agricultural productivity and the intricate web of natural interactions. Rosmarinus, known for its fragrant leaves and culinary applications, secretes allelochemicals that have been found to inhibit the germination and growth of competing plants. Thyme, a herb celebrated for its aromatic qualities, possesses a repertoire of allelochemicals that not only defend its territory but also influence the surrounding vegetation. Artemisia plants, exhibits allelopathic effects that can shape the composition of plant communities. Thyme, a short shrub thriving in challenging environments, completes this quartet of allelopathic botanical agents . The allelopathic interactions between these herbs and shrubs and the wheat and barley crops introduce a layer of complexity to agricultural ecosystems. As global demands for food production escalate, understanding the dynamics of these botanical interactions becomes paramount. How do the allelochemicals released by Rosmarinus, Thyme, Artemisia, and Dryas influence the germination, growth, and yield of wheat and barley plants? Are there potential benefits to harnessing these allelopathic relationships for sustainable agriculture, or do they pose threats to our staple crops? This exploration seeks to unravel the secrets hidden in the soil, uncovering the biochemical dialogues between these plants that shape the very foundation of our food systems. By shedding light on the allelopathic impact of Rosmarinus, Thyme, Artemisia, and Dryas on wheat and barley, we aim to contribute to a more nuanced understanding of the intricate relationships that govern our agricultural landscapes.

2. Materials and Methods

The specimens of Rosmarinus officinalis were individually gathered, air-dried, and subsequently ground using a grinding machine. Each dry sample, weighing 20 g, was dissolved in 100 ml of distilled water (Farhat. etal, 2013). The resulting mixture was then subjected to a 5-hour agitation on a shaker, resulting in an aqueous extract with a concentration of 20%. From this base concentration, dilutions were prepared, namely 16%, 12%, 8%, and 4%, alongside a control (0% distilled water) (Azwanida, 2015).The dilution process adhered to the equation $C2 \times V2 = C1 \times V1$, where V1 represents the initial volume, C1 is the initial concentration, V2 is the final volume, and C2 is the final concentration (Dutta, 2011). For example, to obtain a 16% concentration from the 20% extract, 12 ml of the extract was mixed with 3 ml of distilled water.

(Petri dishes were meticulously cleaned with cotton and acetone, and filter papers were arranged inside each dish. Yes, there are 3 replicates for each treatment. Subsequently, five barley seeds, previously cleaned or sterilized with distilled water, were placed in each dish. Each concentration and the control group contained a total of 15 seeds, divided into 3 replicates of 5 seeds each. Following this, 5 ml of the extract was added to each dish, while distilled water was used for the control group. The dishes were sealed and left in the laboratory for a week, receiving two irrigations during this period to facilitate germination. Measurements of root and stem length were taken using a ruler. All measurements of lengths were in cm A table was compiled with the recorded data for each plant's stem and root. The mean lengths were selected and plotted on a graph sheet to visually represent the extract's impact. All the statistical analysis were performed on SPSS 28, this which included one way analysis of variance and multiple comparisons (IBM Corp., 2021).

3. Results and Discussion

3.1 Results:

3.1.1 Effect on Root Length

The descriptive statistics of the experiment are shown in Table 1. A one-way analysis of variance (ANOVA) was conducted using SPSS to assess the impact of different experimental conditions on barley root measurements (Sahu and Sahu, 2016). The results revealed a significant difference in barley root measurements among the treatments, as evidenced by a significant F-statistic ($F(4, 10) = 15.58$, $p < .001$). The Between Groups comparison, with a sum of squares of 38.85 and 4 degrees of freedom, resulted in a mean square of 9.71. Conversely, the Within Groups comparison, with a sum of squares of 6.24 and 10 degrees of freedom, produced a mean square of 0.62. The Total sum of squares, combining both Between and Within Groups variability, was 45.09 with 14 degrees of freedom.

Table 1. The descriptive statistics of barley root measurement according to the experiment conditions.

	barley root measurement (cm)					
***Rosmarinus officinalis* extract**	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
control	3	4.65	.786	.454	2.70	6.60
16% conc.	3	.25	.433	.250	-.83	1.33
12% conc.	3	1.42	.629	.363	-.15	2.98
8% conc.	3	.33	.577	.333	-1.10	1.77
4% conc.	3	2.17	1.258	.726	-.96	5.29
Total	15	1.76	1.795	.463	.77	2.76

These findings suggest that the variability in Barley root measurements is not merely due to chance and can be attributed to the differences in experimental conditions. Further post-hoc analyses to identify specific group differences was conducted.

The results of multiple comparisons for the dependent variable "Barley root measurement" under different experimental conditions were analysed using the LSD (Least Significant Difference) test to compare the mean differences between various concentration levels of an experimental condition and a control group (Meier, 2006). The significance level is set at 0.05. LSD results were as follows:

16% concentration: The mean difference in Barley root measurement between the control group and the 16% concentration group is 4.40 (95% CI =2.96 to 5.84), which is statistically significant ($p < 0.001$).

12% concentration: The mean difference between the control and the 12% concentration group is 3.23 (95% CI=1.80 to 4.67), showing statistical significance ($p < 0.001$).

8% concentration: The mean difference in Barley root measurement between the control and the 8% concentration group is 4.32 (95%=2.88 to 5.75), with statistical significance ($p < 0.001$).

4% concentration: The mean difference between the control and the 4% concentration group is 2.48 (95% CI=1.05 to 3.92), and this difference is statistically significant ($p = 0.003$).

In addition, Figure 2 is showing that increasing the concentration of Rosmarinus officinalis extract correlates with a greater disparity in barley root length compared to the control. In other words, higher concentrations lead to shorter root lengths. This correlation was significant as r was equal to 0.65. according to the different concentration of the Rosmarinus officinalis extract.

Figure 1. One of the repeats of the experiment showing the different lengths of the roots and stems of barley plant

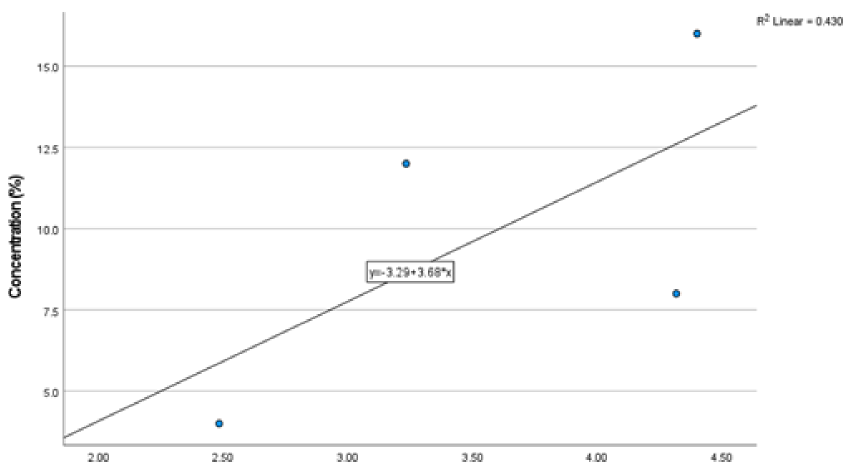

Figure 2. Shows increasing the concentration of Rosmarinus officinalis extract correlates with a greater disparity in barley root length compared to the control. In other words, higher concentrations lead to shorter root lengths.

3.1.2 Effect on stem length

Table 2 shows the descriptive statistics on barley stem measurement according to the experiment conditions. A one-way analysis of variance (ANOVA) was conducted to examine the impact of different experimental conditions on barley stem measurement. The analysis revealed a significant difference in barley stem measurement among the experimental conditions, as indicated by a highly significant F-statistic ($F(4, 10) = 45.777$, $p < .001$). The Between Groups comparison, with a sum of squares of 29.786 and 4 degrees of freedom, resulted in a mean square of 7.446. Conversely, the Within Groups comparison, with a sum of squares of 1.627 and 10 degrees of freedom, produced a mean square of 0.163. The Total sum of squares, combining both Between and Within Groups variability, was 31.412 with 14 degrees of freedom.

The Multiple Comparisons table explores the impact of different experimental conditions on the dependent variable which is barley stem measurement. The LSD test was employed to assess the significance of mean differences between each experimental condition and the control group, along with the confidence intervals. The results were as follows:

16% concentration: The mean difference in RM2 between the control group and the 16% concentration group is 3.77, and this difference is statistically significant ($p < 0.001$). The 95% confidence interval ranges from 3.03 to 4.50.
12% concentration: The mean difference between the control and the 12% concentration group is 3.43, with statistical significance ($p < 0.001$). The 95% confidence interval ranges from 2.70 to 4.17.

Table 2. The descriptive statistics on barley stem measurement according to the experiment conditions.

`	barley root measurement (cm)					
Rosmarinus officinalis extract	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	**Upper Bound**
control	3	4.27	.252	.145	3.64	4.89
16% conc.	3	.50	.000	.000	.50	.50
12% conc.	3	.83	.289	.167	.12	1.55
8% conc.	3	1.42	.804	..464	-.58	3.41
4% conc.	3	2.92	.144	.083	2.56	3.28
Total	15	1.99	1.498	.387.	1.16	2.82

8% concentration: The mean difference in RM2 between the control and the 8% concentration group is 2.85, and this difference is statistically significant ($p < 0.001$). The 95% confidence interval is from 2.12 to 3.58.
4% concentration: The mean difference between the control and the 4% concentration group is 1.35, which is statistically significant ($p = 0.002$). The 95% confidence interval ranges from 0.62 to 2.08.

These comparisons are reciprocated for the inverse relationships (16%, 12%, 8%, and 4% concentration groups compared to the control group). In all cases, the mean differences are deemed significant at the 0.05 level.
In addition, Figure 3 shows that increasing the concentration of Rosmarinus officinalis extract correlates with a greater disparity in barley stem length compared to the control. In other words, higher concentrations lead to shorter root lengths. This correlation was significant as r was equal to 0.94.

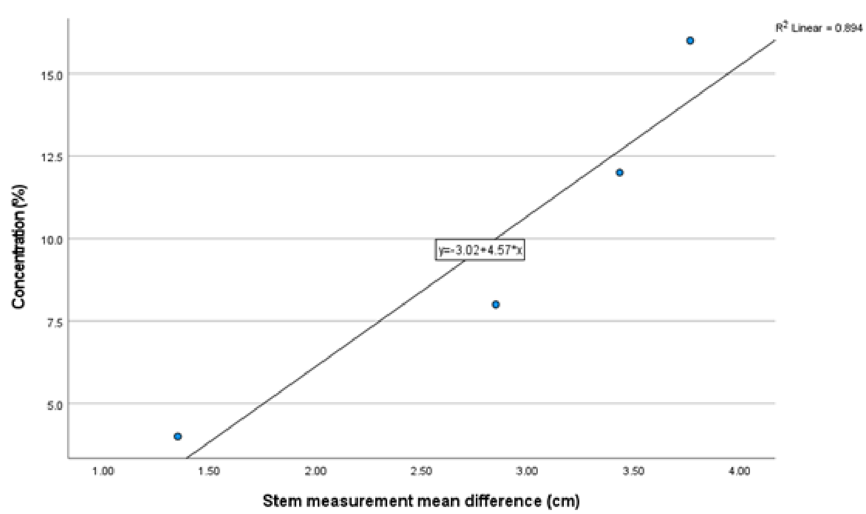

Figure 3. shows the Increasing the concentration of Rosmarinus officinalis L. extract correlates with a greater disparity in barley stem length

3.2. Discussion

Root and stem measurements, using these indicators as proxies for overall plant growth. The significance of this research lies in its implications for agricultural practices in Libya and other regions of the Middle East and North Africa, such as *Rosmarinus officinalis* L. , might affect the growth and development of barley crops, an essential crop in the region (Ullrich, 2010).

The results of the experiment, as revealed through the ANOVA and LSD tests, suggested an inhibitory impact of Rosmarinus officinalis Weed extract on the growth of barley plants under experimental conditions. This is in accordance with various studies (Uludag, et. 2006 and Coskun et al. 2022). These differences indicate that the presence of Rosmarinus officinalis Weed extract, under various concentrations and conditions, distinctly influenced the growth parameters of barley plants. The comprehensive understanding gained from these statistical analyses suggests that the extract has an overall impact on the growth of barley. However, it is important to know the impact of this medicinal plant in under real conditions because some studies suggested that Rosmarinus species have a positive impact as an effective antifungal and herbicidal agent (Kaab, et al. 2019).

However, the argument can be extended to propose that the existence of certain medicinal plants, like Rosmarinus officinalis, within the barley crop field might pose a significant risk to the overall development and growth of the barley plants. Medicinal plants often contain bioactive compounds that, while beneficial for human health, can exert allelopathic effects on neighboring plants (Zheljazkov, et al. 2021). In the context of this experiment, the presence of Rosmarinus officinalis Weed extract appears to have influenced the growth of barley, potentially hindering its development under certain concentrations

The results contribute valuable insights for further research in crop science and agronomy. The emphasis on considering specific conditions for optimal plant growth and development is crucial for designing sustainable and productive agricultural practices. If, indeed, the presence of Rosmarinus officinalis Weed extract has a discernible impact on barley growth, it raises questions about the co-cultivation or rotation of medicinal plants with traditional crops (Brooker, et al. 2015). Farmers and researchers need to carefully consider these interactions to ensure the health and productivity of agricultural systems.

4. Conclusion

The study "Allelopathic Influence of Rosmarinus officinalis Aqueous Extracts on Barley Plants" investigated the inhibitory effects of rosemary extracts on the growth of barley. By preparing aqueous extracts *Rosmarinus officinalis* L. at various concentrations, the research aimed to understand the allelopathic interactions between rosemary and barley, a critical crop in many regions, including the Middle East and North Africa.

The results demonstrated that increasing concentrations of Rosmarinus officinalis extracts significantly inhibited the growth of barley roots and stems. Statistical analyses, including one-way ANOVA and LSD tests, confirmed the substantial impact of these extracts. Higher concentrations led to shorter root and stem lengths, indicating a negative correlation between the concentration of rosemary extract and barley growth. This research provides crucial insights for agricultural practices, particularly in regions where barley is a staple crop. The findings suggest that the presence of Rosmarinus officinalis, whether through direct contact or residual allelochemicals in the soil, could hinder barley growth. This has significant implications for crop management and the strategic planning of crop rotations and co-cultivation practices. However, while the inhibitory effects of rosemary extracts were evident under controlled experimental conditions, further research is needed to explore these interactions in real-world agricultural settings. Understanding the broader ecological impacts and potential benefits, such as antifungal and herbicidal properties of Rosmarinus officinalis, is essential for developing sustainable agricultural systems.

In conclusion, the allelopathic influence of Rosmarinus officinalis presents both challenges and opportunities for crop science and agronomy. By shedding light on these intricate plant interactions, this study contributes to a more nuanced understanding of how to optimize agricultural productivity while maintaining ecological balance.

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