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Allelopathic Effects of *Artemisia judaica* and *A. campestris* Aqueous Extracts on *Lactuca sativa* L Seeds Germination

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التأثيرات الأللوبياثية للمستخلصات المائية لنباتي *Artemisia judaica* و *Artemisia campestris* على إنبات بذور نبات الخس *(Lactuca sativa L.)*

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Abstract:

This study investigated the allelopathic potential of aqueous extracts from two Libyan *Artemisia* species, *Artemisia judaica* and *Artemisia campestris*, on the seed germination and germination rate of *Lactuca sativa* L. Aerial parts of the plants were collected during the flowering stage in February 2020 from Zintan, Libya. Qualitative phytochemical analysis of the aqueous extracts revealed the presence of tannins, flavonoids, and saponins in both species, while phlobatannins and terpenoids were absent. A bioassay demonstrated that both *A. judaica* and *A. campestris* extracts exhibited significant allelopathic activity, inhibiting *Lactuca sativa* seed germination and decrease the germination rate (GR) in a dose-dependent manner. Complete inhibition of germination (100% IP) was observed at concentrations of 10 mg/ml and 20 mg/ml for both species. The findings suggest that the observed allelopathic effects are likely due to the identified phytochemical constituents, and that these *Artemisia* species could serve as promising sources for natural herbicides in agricultural weed control.

Keywords: Allelopathy, *Artemisia Judaica*, *Artemisia campestris*, *Lactuca sativa*, Phytochemicals, Natural herbicides.

الملخص

تناولت هذه الدراسة التأثرات والإمكانات الأليلوباثية للمستخلصات المائية من نوعين من الشيح الليبي، الشيح العراقي (*Artemisia judaica*) وشيح الحقول (*Artemisia campestris*) ، على إنبات البذور ومعدل الإنبات لنبات الخس (*Lactuca sativa L.*). تم جمع الأجزاء الهوائية للنباتات خلال مرحلة الإزهار في فبراير 2020 من الزنتان، ليبيا. كشف التحليل الكيميائي النباتي النوعي للمستخلصات المائية عن وجود التانينات والفلافونويدات والصابونينات في كلا النوعين، بينما كانت الفلوباتانينات والتربينويدات غائبة. أظهر الفحص الحيوي أن مستخلصات كل من الشيح العراقي وشيح الحقول أبدت نشاطاً أليلوباثياً كبيراً، حيث ثبطت إنبات بذور الخس وقللت من معدل الإنبات (GR) بطريقة تعتمد على الجرعة. لوحظ تثبيط كامل للإنبات (IP 100%) عند تراكيز 10 مجم/مل و20 مجم/مل لكلا النوعين. تشير النتائج إلى أن التأثيرات

الأليلوباثية المُلاحظة ترجع على الأرجح إلى المكونات الكيميائية النباتية المُحددة، وأن أنواع الشيح هذه يمكن أن تكون بمثابة مصادر واعدة لمبيدات الأعشاب الطبيعية في مكافحة الأعشاب الضارة الزراعية.

الكلمات المفتاحية: الأليلوباثي، الشيح العراقي، شيح الحقول، الخس، المواد الكيميائية النباتية، مبيدات الأعشاب الطبيعية.

Introduction

Artemisia judaica and *A. campestris* are not just any plants; they are common perennial shrubs deeply rooted in the semi-arid landscapes of the Middle East [1]. For centuries, these species have held a significant place in traditional medicine, particularly among Bedouin communities who have long utilized them in various forms, from soothing hot infusions to refreshing cold beverages [2]. This traditional wisdom hints at the rich tapestry of bioactive compounds these plants possess, compounds that are increasingly being recognized for their diverse biological activities, including their potential to influence the growth of other plants.

The scientific community has long been fascinated by the intricate chemical interactions between plants, a phenomenon known as allelopathy. This natural process involves the release of biochemicals, or allelochemicals, from one plant that can either inhibit or stimulate the growth and development of neighboring organisms [3]. In an era where environmental sustainability is paramount, allelopathy offers a compelling alternative to synthetic herbicides, which often carry ecological burdens [4-5]. Indeed, the quest for eco-friendly weed management strategies has brought the allelopathic potential of various *Artemisia* species into sharp focus. Numerous studies have highlighted their remarkable ability to suppress the germination and growth of a wide array of weeds and even certain crops [6-8].

The secret behind these effects often lies in the complex cocktail of allelochemicals, including phenolic compounds, flavonoids, and terpenoids [9-10]. These natural compounds can subtly, yet powerfully, interfere with crucial physiological processes in target plants, disrupting hormone activity, altering membrane permeability, and hindering nutrient uptake [11]. Given this rich background, our study embarks on a focused investigation into the allelopathic potential of aqueous extracts derived from *Artemisia judaica* and *Artemisia campestris* on the germination of *Lactuca sativa*. By delving deeper into these interactions, we aim to contribute valuable insights that could pave the way for developing novel, natural herbicides, fostering a more sustainable approach to agricultural weed control.

Materials and Methods

Plant collection

The aerial parts of *Artemisia judaica* and *Artemisia campestris* collected during their flowering stage in February 2020. *A. campestris* was collected from Zintan at geographical coordinates N31°57’21.19’’; E12°13’6.35’’, while the geographical coordinates for *A. judaica* collection were N29°33′44.97″ E10°14′15.13 from AL-Hmada. The plants were Identified by Dr. Ali Filaly Faculty of Agriculture – University of Zintan.

Extraction Preparation

Both *A. judaica* and *A. campestris* aqueous extracts were prepared according to the procedures described by [12]. The aerial parts of each species were cleaned, air-dried in the shade for ten days, and then ground into a fine powder. An amount 100g of the plant’s materials macerated in one Litter of distilled water and kept at room temperature for twenty-four hours (24h) in dark, with intermittent shaking. The extract was then filtered through a fine mesh cloth, followed by filtration through Whatman No. 1 filter paper to remove any impurities and insoluble materials. The resulting aqueous extract was then concentrated using an oven to evaporator at a controlled temperature (40°C) to obtain a crude extract. This crude powder extracts stored at 4°C until further use. For the bioassay, stock solutions of the extracts were prepared by dissolving the dry powder in distilled water to achieve the desired concentrations (5, 10, 15, and 20 mg/ml).

Phytochemical Analysis

To qualitative chemical constituents, present in the aqueous extracts of the two Artemisa species, a series of standard chemical procedures were used, as described by [13]. Specifically, tests were conducted to detect the presence of tannins, saponins, flavonoids, terpenoids, and phlobatannins. For tannin detection, the ferric chloride test and gelatin test were performed. Saponins were identified using the froth test. Flavonoids were detected using the alkaline reagent test and the lead acetate test. The Salkowski test and Liebermann-Burchard test were used for terpenoids, while the ferric chloride test was also employed for phlobatannins. These qualitative analyses provided insights into the general phytochemical profile of the prepared extracts.

Allelopathic Bioassay

The allelopathic potential of the aqueous extracts on seed germination and germination rate were assessed using a bioassay method adapted from [14]. *Lactuca sativa* L. (lettuce) seeds were used as the test plant due to their

sensitivity to allelochemicals and their widespread use in allelopathy studies. The experiment was conducted in a controlled condition for ten days. Four different concentrations of the aqueous extracts (5, 10, 15, and 20 mg/ml) were prepared. For the negative control distilled water was used. For each treatment, a thirty seeds of *Lactuca sativa* seeds were sited in Petri dishes lined with filter paper, and then 5 ml of the respective extract concentration or distilled water were added. Each treatment was replicated three times. The Petri dishes then incubated at a constant temperature 25°C with a 12-hour light/12-hour dark. Only distilled water were added when needed. Seed germination was recorded daily, and a seed was considered germinated when the radicle emerged and was at least 2 mm long. The inhibition or germination percentages and germination rate were subsequently calculated using the equations provided by [15]. The germination percentage (GP) was calculated as (Number of germinated seeds / Total number of seeds) * 100. The germination rate (GR) was calculated using the formula: Germination Rate = Σ (Ni / Di), where Ni is the number of seeds germinated on day i, and Di is the number of days after sowing.

Results

Phytochemical Analysis

The qualitative phytochemical analysis of the aqueous extracts from *A. judaica* and *A. campestris* as summarized in (Table 1) showed the presence of several key compounds. Both species tested positive for tannins, flavonoids, and saponins. Nevertheless, phlobatannins and terpenoids were absent from the aqueous extracts of both *Artemisia* species.

Table 1: Phytochemical constituents of water extract of studied species.

Compounds	Tannins	Phlobatannins	Flavonoids	Saponins	Terpenoids
A. judaica	+	-	+	+	-
A. campestris	+	-	+	+	-

Compound present (+), compound absent (-)

Allelopathic effects on Seed Germination and Germination Rate

The results of the allelopathic effects of the aqueous extracts from *A. judaica* and *A. campestris* on the seed germination of Lactuca sativa was collected from the bioassay results (Fig 1 and Fig 2). showed inverse relationship was observed between the concentration of the extracts and the germination percentage (GP) of the seeds. As the extract concentrations increased, the germination percentage decreased. Specifically, both *A. judaica* and *A. campestris* extracts demonstrated complete inhibition of seed germination (IP) at concentrations of 10 mg/ml and 20 mg/ml, respectively. This indicates a potent allelopathic effect at higher concentrations. (Fig 3) showed the effects of the water extract on germination rate (GR) the germination rate showed in (Fig. 3) was decrease with the increase of the extracts concentrations of both species

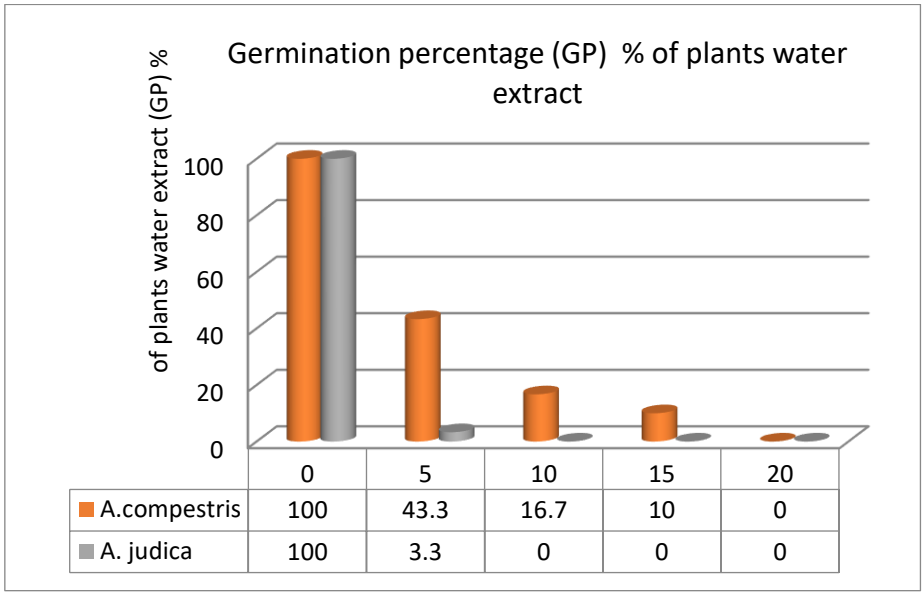

Fig. (1) The GP values % of plants extract of plants extract .

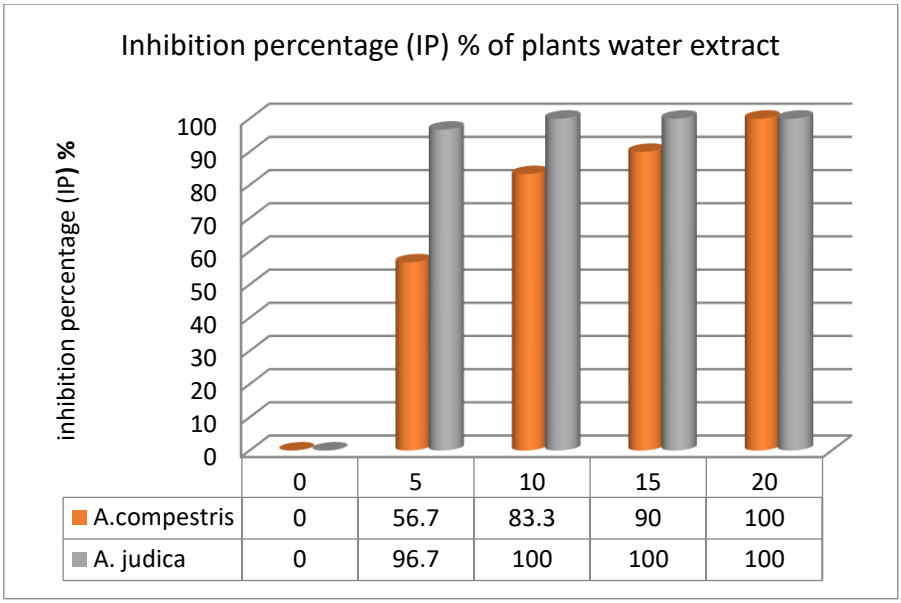

Fig. (2) The IP values % of plants extract of plants extract.

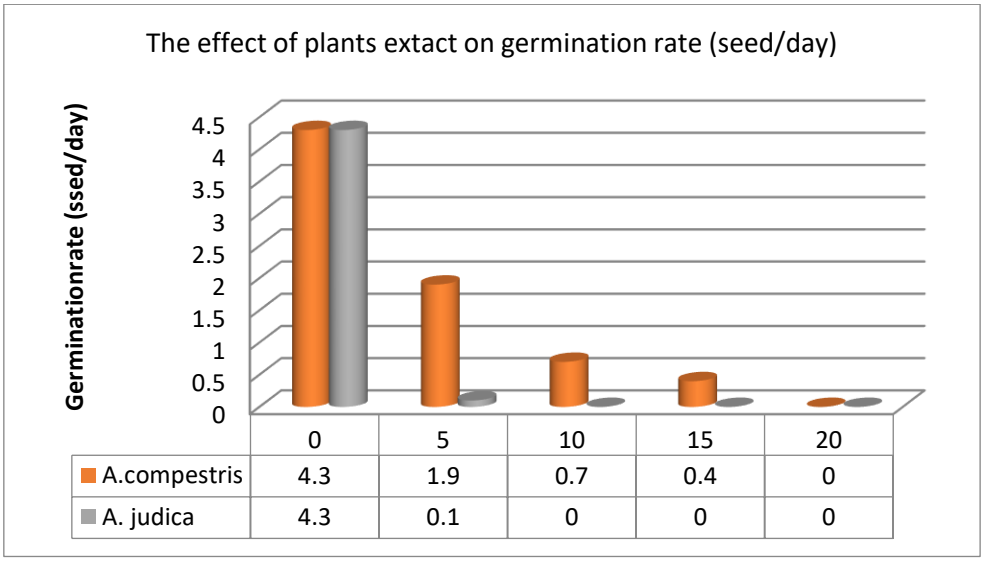

Fig. (3) The effect of plants extract on germination rate (seed/day).

Discussion

The observed allelopathic effects of *Artemisia judaica* and *Artemisia campestris* aqueous extracts on *Lactuca sativa* seed germination are consistent with the known phytotoxic properties of various *Artemisia* species. The phytochemical analysis revealed the presence of tannins, flavonoids, and saponins in both *Artemisia* extracts. These compounds are well-documented for their diverse biological activities, including allelopathy [16]. The absence of phlobatannins and terpenoids in the aqueous extracts suggests that the observed inhibitory effects are primarily attributable to the identified phenolic compounds and saponins.

The inverse relationship between extract concentration and germination percentage strongly supports the dose-dependent nature of allelopathic inhibition. Complete inhibition of *Lactuca sativa* seed germination at higher concentrations (10 mg/ml and 20 mg/ml) for *Artemisia judaica* and *Artemisia campestris* respectively highlights the potent allelopathic potential of these *Artemisia* species. This is further supported by germination rate (GR) data: for *A. campestris*, GR decreased from 4.3 at 0 mg/ml to 0.0 at 20 mg/ml, with intermediate values of 1.9, 0.7, and 0.4 at 5, 10, and 15 mg/ml respectively. In contrast, *A. judaica* showed an even stronger allelopathic effect, with GR dropping sharply from 4.3 at 0 mg/ml to 0.1 at 5 mg/ml, and complete inhibition (0.0 GR) observed from 10 mg/ml onwards.

These findings are Compatible with previous research demonstrating significant germination inhibition (IP) by *Artemisia* extracts on various target species [14]. For instance, studies have shown that essential oils from *Artemisia annua* and *Artemisia verlotiorum* can significantly inhibit seed germination of weeds like *A. retroflexus* [4]. Similarly, *Artemisia scoparia* has been reported to exhibit phytotoxic effects on various seeds [17]. The greater activity of *A. judaica* compared to *A. campestris*, as suggested by the abstract and supported by the GR data, could be attributed to variations in the concentration or specific types of allelochemicals present in their respective aqueous extracts, a phenomenon observed in other *Artemisia* species where allelopathic potential can vary with geographical location and environmental factors [7]. This aligns with earlier findings that different *Artemisia* species and even populations within the same species can exhibit varying degrees of allelopathic activity due to differences in their chemical profiles [8].

Allelopathy, mediated by secondary metabolites released from plants, plays a crucial role in plant–plant interactions and can influence agricultural ecosystems by suppressing weed growth [18]. The potent inhibitory effects observed in this study suggest that aqueous extracts of *A. judaica* and *A. campestris* could be promising candidates for developing natural herbicides. This aligns with the growing interest in sustainable agricultural practices and the reduction of synthetic chemical inputs [19]. The use of natural herbicides derived from allelopathic plants is gaining traction as an environmentally friendly alternative to synthetic pesticides [6]. The concept of utilizing allelopathy for weed control has been explored for decades [3,11], and our findings provide further evidence for the practical application of *Artemisia* species in this regard. Further research is warranted to isolate and characterize the specific allelochemicals responsible for these effects and to evaluate their efficacy and safety in field conditions, potentially leading to the development of novel bioherbicides.

Conclusion

This study confirms the strong allelopathic potential of Artemisia judaica and Artemisia campestris aqueous extracts in inhibiting Lactuca sativa seed germination in a dose-dependent manner. The presence of tannins, flavonoids, and saponins likely contributes to this phytotoxic activity. These findings support the potential use of Artemisia extracts as natural bioherbicides in sustainable agriculture. Further research is needed to isolate the active compounds and assess their effectiveness under field conditions.

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