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The Allelopathic Effects of *Artemisia herba-alba* Asso. Aqueous Extracts on Seed Germination and Seedling Development of *Ceratonia siliqua* L.

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الآثار الأليلوباثية للمستخلصات المائية للشيح *Artemisia herba-alba* Asso. على إنبات البذور ونمو البادرات للخروب *Ceratonia siliqua* L.

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Abstract

This study aims to investigate the allelopathic effects of *Artemisia herba-alba* Asso. aqueous extracts on the germination of *Ceratonia siliqua* L. seeds and seedlings. Three concentrations 100%, 50 %, 25%, and control (distill water) were used for testing. Seeds and seedlings of *Ceratonia siliqua* were exposed to above-mentioned concentrations. One-way ANOVA test was adopted to examine the effect of these treatments on germination rate and root length for the seeds of *Ceratonia siliqua*. Our results showed no significant differences between the concentrations on germination rate as the F-Value and P-Value were 2.88 and 0.08 respectively. However, high significance occurred after testing concentrations against root length with F-value reaching (139.89). Tukey HSD (Honestly Significant Difference) test showed that highest significance in means was imposed by both (100 % and 50 %) levels of concentration on root length. It also shows a strong inverse relation between root length and concentrations levels reflected in R-squared value of (0.93). The treatments affected *Ceratonia siliqua* roots by inhibiting their length significantly (F-Value: 21.1). In conclusion, the increase of concentration levels result in the inhibition of mainly root length. That may explain the law survival of *Ceratonia siliqua* seedlings in regions where *Artemisia herba-alba* grow. However, further studies are required to investigate other factors.

***Keywords:** allelopathy; Artemisia herba-alba; aqueous extracts; root length.*

الملخص

تهدف هذه الدراسة للتحقيق في الخصائص الأليلوباثية لنبات الشيح *Artemisia herba-alba* Asso. وذلك من خلال تقييم تأثير مستخلصاته المائية على نسب الإنبات وعلى بادرات نبات الخروب *Ceratonia siliqua L.* تم تحضير المستخلصات بثلاثة تركيزات (25%، 50 %، 100%) وشاهد (ماء مقطر). تم معاملة بذور وبادرات الخروب بهذه المعاملات. تم تبني اختبار تحليل التباين (ANOVA) بوجود متغير واحد لاختبار تأثيرات هذه المعاملات على معدل الإنبات وطول الجذور الناتجة في بادرات الخروب. لم تظهر نتائجنا فروق معنوية بين هذه المعاملات على نسب الإنبات إذ أن قيم F-Value و P-Value كانتا 2.88 و 0.08 على التوالي. غير أنه تم ملاحظة معنوية عالية عند مقارنة طول الجذور بالتركيزات المختلفة حيث وصلت قيمة F-Value إلى (139.89). اختبار توكي Tukey HSD (الاختلافات المعنوية الصادقة) أظهر مستوى عالٍ في متوسطات أطوال الجذور نتيجة للتركيزين (50% و 100%). الاختبار أظهر أيضاً علاقة عكسية قوية بين مستويات التركيز وطول الجذور حيث انعكست في قيمة (R^2) لتصل 0.93. جذور البادرات شهدت تثبيطاً عالي المعنوية (F-Value: 21.1). استنتاجاً، زيادة التركيز تؤدي بشكل

أساسي لتثبيط جذور الخروب. هذا قد يفسر انخفاض عدد بادرات الخروب الناجية في المناطق التي ينمو بها نبات الشيح، إلا أنه يجب دراسة كل العوامل الأخرى التي تساهم في هذه الظاهرة.

الكلمات الدلالية: الأليلوباثية، الشيح، المستخلصات المائية، طول الجذور.

1. Introduction

One of the most fundamental driving forces in forest ecology, and eventually in forest dynamics and biodiversity, is plant-to-plant relationships (Dunnett & Grime, 1999; Escudero *et al.*, 2000; and Brooker, 2006). Allelopathy in plants can be defined as the effects of one plant on other plant through the release of chemical compounds in the environment (Rice, 1984; Hussain & Khan, 1988; and Mallik, 2008). Therefore, the natural regeneration of certain species in forest ecosystems maybe affected by allelopathy. Several studies have reported the inhibitive behavior of different plants on the germination of their seeds or on the seeds of other species, which results in the alternation of seed bank viability in forests as it discussed in the current context. In arid and semi-arid regions of the world, it is important to consider all factors that may affect the plant community succession, composition and dynamics since the competition between plants is very high due to the limited water and nutrition resources in such parts of the planet (Escudero *et al.*, 2000). Chemical microenvironment constitutes determinant factors that promote or inhibit seed germination are proved to be produced by a number of species in arid and semi-arid regions (Long *et al.*, 2015).

Artemisia herba-alba Asso (white wormwood) is a perennial shrub occurs generally on the dry steppes of the Mediterranean regions in Northern Africa (Morocco, Tunisia, Algeria, and Libya), Western Asia (Arabian Peninsula) and Southwestern Europe (www.theplantlist.org). It is a micro-chamaephyte, perennial plant in which the living parts of aerial shoots become very reduced in summer, 20-40 *cm*, aromatic, tomentose, greyish, easily uprooted owing to its superficially ramified roots. Heads sessile, oblong, 3-4 *mm*, 2-4 flowered (IUCN, 2005). The species belongs to Asteraceae family and has other Synonyms namely: *A. aethiopica* L., *A. aragonensis* Lam., *A. lippii* Jan ex Besser, *A. ontina* Dufour, and *Seriphidium herba-alba* Soják. It is used as an antiseptic and antispasmodic in herbal medicine (Yashphe *et al.*, 1987). In terms of its chemical composition, *A. herba-alba* has five main substances: α-thujone; campholene aldehyde; 2, 4-Hexadiene, 2, 3-dimethyl-; *Artemisia triene and Sabinene* (Sbayou *et al.*, 2014). A very recent research conducted by Arroyo *et al.* (2016) cited most important studies that investigated the allelopathic properties of *A. herba-alba* and concluded that it is a remarkably allelopathic species, especially with the increase of concentration. According to Arroyo *et al*. (2016), the application of *A. herba-alba* aqueous extract reduced seedling emergence from the seed bank by 50%. Other authors reported similar results regarding the exhibition of allelopathy by many herbaceous, aromatic shrubs and trees species in regions that have similar environmental conditions to the Mediterranean ecosystems (Thompson, 2005; and Scognamiglio *et al*., 2013). It seems that most species of the genus *Artemisia* have general positive tendency towards allelopathy, yet with different extents between its species. Examples of this tendency are documented by several recent studies.

Elshatshat (2010) reported a proportional relationship between seven concentration levels of *Artemisia herba-alba* aqueous extract and the inhibition level on Tomato and Wheat seed germination. Salhi *et al.* (2011) found that the aqueous root extract of *Artemisia herba-alba* has no significant effect on the germination of *Avena fatua* L. and *Polygonum convolvulus* L, whereas the aerial parts aqueous extract showed higher significance effect on same measured parameters. Abd El-Fattah *et al*. (2011) examined the allelopathy of *Artemisia princeps* extracts (3.75 and 5% *w/v*) and they were phytotoxic to germination of wheat. The reduction in the germination percentage reached 15 to 30% by treatment with *A. princeps* after 8 days of treatment. Bataw and Hughes (2013) investigated the effect of *Artemisia herba-alba* on common bean and concluded that the 4% aqueous extract of shoots contain allelopathic chemicals and phytotoxic compounds that may lead to an increase in unsaturated and saturated fatty acids in germinated common bean seedlings. Other results obtained by Tilaki *et al.* (2013) showed that increase in essential oil concentration reduced root lengths, seed germination percentage and vigor index of *Agropyron desertorum* and *Agropyron cristatum.*

Understanding the role of allelopathy exhibited by plants in forest ecosystems may represent a potential tool for management (Cummings *et al.*, 2012). Weed management, seed bank and seedling establishment and growth can be controlled by inducing or, alternatively, reducing naturally produced chemical compounds derived from local spices (Caboun & Jhon, 2015).

To make a brief summary of what we discussed above, it is important indeed to understand the role of allelopathy phenomenon. Such importance should be a priority in regions where natural vegetation is degraded like what we have in Libya. Studies about the role of allelopathy in forest ecosystems development are, in fact, rare (Elshatshat, 2010). For these reasons, we present this study to examine the effect of *Artemisia herba-alba* aqueous extract on a vital forestry species *Ceratonia siliqua* L.

C. siliqua from Caesalpiniaceae family It is one of the most important species. It is used locally for the production of honey and the Carob syrup. It is also a good source of food for wild animals and demotic livestock. It faces a decline in the production of seedlings despite the massive amount of seeds produced by mother trees in natural habitats. Over-grazing could be a major reason for the loss of fresh seedlings but in some inaccessible locations, it is hardly to notice any natural regeneration. Hence, comes the present study to observe the possible role of allelopathy on this species and to, further, understand about the action of *Artemisia herba-alba* in nature.

2. Material & Methods

2.1. Plant Material Collection

As it presented in Figure (1) below, *A. herba-alba* samples were from the south of Al-Jabal Al-Akhdar, Al-Mashal (32° 30.467' N, 21° 44.075' E) in July 2017. We collected only areal parts. *C. Siliqua* seeds were collected from Bulgray (32° 43.505' N, 21° 42.455'E) in August 2016.

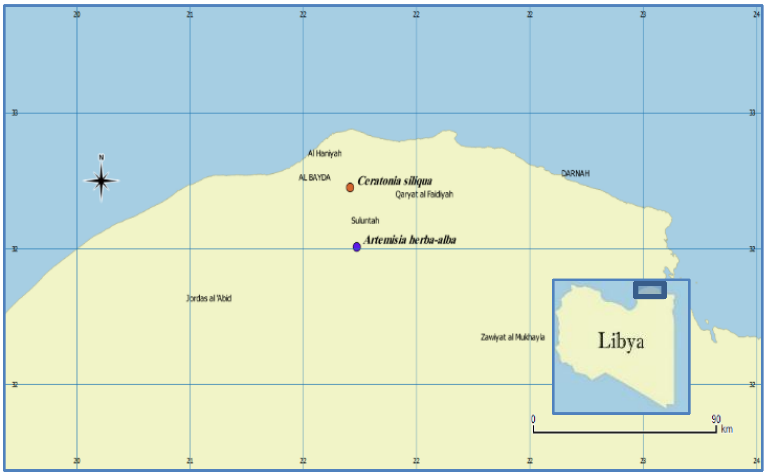

Figure 1. Locations of samples.

2.2. Plant Material Preparation

2.2.1. Preparing the aqueous extract of Artemisia herba-alba

After the collection of the plants, they were dried in a shady place at room temperature for ten days. At the forestry department laboratory of Omar Al-Mukhtar University, the dried areal parts (leaves and stems) were ground into powdered form. Then 50 *g* of the powder were diluted into 500 *ml* of distilled water (Bajalan *et al*., 2013). Next to that, this mixture was left on a Shaker for 24 *hrs* in room temperature at speed of 120 *rpm* (Ghorbani *et al*., 2008). A four-folded cotton fabric was used as a filter to separate rough solid particles from solution, and then it was centrifuged with the speed of 2000 *rpm* for 15 *minutes* (Bajalan *et al*., 2013). Three concentrations of solutions were prepared based on volume/volume percent (*v/v.* %) except for the basic solution 100 % which was a weight/ volume percent (*w/v.* %) (Elshatshat, 2010). Three concentrations were prepaid (25, 50, and 100%) in addition to the distilled water as a control.

2.2.2. Preparing Germination and Seedling Experiments

The seeds of *C. siliqua* were cleaned and then floating test was implemented. To break seeds dormancy and deviate its effect on germination, a chemical treatment was applied. According to (Bostan, and Kilic, 2014), treating *C. siliqua* seeds with Sulphuric acid (95 %) for 20 *minutes* would result in high germination percentage reaching (95–99 %). A test was conducted to confirm these results to be used in this experiment. Results of the current study was very similar and, hence, used in this study. Completely Randomized Design CRD was implemented. 80 treated seeds were randomly collected and randomly distributed on 16 Petri dishes (90 mm diameter), five seeds per dish. Each treatment concentration was applied on four Petri dishes (Four replicates). The parameter of interest in this part of the study was the germination rate after the application of (distilled water, 25, 50, and 100% of *Artemisia herba-alba*). All dishes were placed in a low temperature incubator at 21 ^{o}C for eight days (Abd El-Fattah *et al.*, 2011; Al-Watban & Salama, 2012; and Bataw & Hughes, 2013). Percentage of germination for each dish was calculated and the root length for germinated seeds was measured. For the evaluation of growth parameters (total length, root length, and number of leaves), 12 early young seedlings of *C. siliqua* were randomly measured. Each three seedlings were irrigated by the same concentration level. Root length, stem length and number of leaves were recorded. One-way ANOVA ($P < 0.05$) was carried out using Minitab 18 (Trail Licensed Version, 2017) to compare the germination among treatments.

3. Results and Discussion

3.1. Seed Germination

The germination started in the control treatment in 48 hours from the beginning of the trial reaching 100% in all four dishes. Observations were recorded every 48 *hrs* and the germination was tested in eight days (Abd El-Fattah *et al.*, 2011; and Al-Watban & Salama 2012). The analysis of variance showed that there are no significant differences between the effects of *A. herba-alba* aqueous extracts on the germination of *C. siliqua* seeds after eight days of the experiment. Similar findings on the germination of *C. siliqua* has been reported by (Bataw and Hughes, 2013; and Tilaki *et al*., 2013).

3.2. Root Length of Germinated Seeds

The measurements taken by the end of the germination trials showed a very clear significan as shown in Table (1). Tukey HSD (Honestly Significant Difference) test revealed that all treatments exhibited high significance between all pair-wised comparisons (Arroyo *et al*., 2016). As it appears from Table (1), subjecting seeds to higher concentration results in the inhibition of root length. These findings confirm what has been demonstrated by (Elshatshat, 2010; Bataw & Hughes, 2013; and Tilaki *et al.*, 2013).

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Table 1. Effects of *Artemisia herba-alba* aqueous extracts on germination & root length of *C. siliqua*. α = 0.05

Concentration (%)	Means	
	Germination rate (%)	**Root length (*cm*)**
Control	100	12.66[a]
25 %	100	9.11[b]
50 %	100	7.015[c]
100 %	65	4.07[d]
F	2.88 n/s	139.89**
HSD	-	0.432

Means within columns followed by different letters are significantly different according to Tukey (HSD) P<0.05.

Noticeably, there was a systematic and proportional pattern relates the decline in root length of *C. Siliqua* and the increase in the concentration of *Artemisia herba-alba* aqueous extracts. The function between both variables is statistically illustrated in Figure (2). The coefficient of determination value (R^2) reached to 0.93 and the model of regression can be expressed by bellow given equation:

$$R_L = 12.57 - 14.44\,C + 5.967\,C^2 \qquad \text{(1)}$$

Whereas: R_L is root length and C is concentration value.

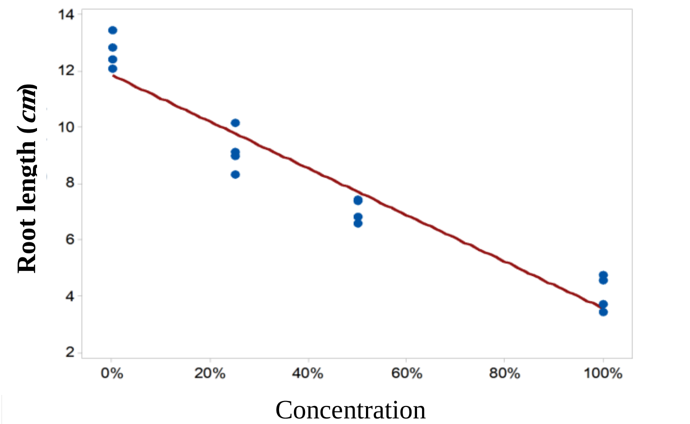

Figure 2. Analysis of regression between root lengths of *C. Siliqua* seedlings and concentrations of *Artemisia herba-alba* aqueous extracts

3.3. Inhibitory effect of *A. herba-alba* on seedling growth and development

One-way ANOVA (P < 0.05) was carried out using Minitab 18 (Trail Licensed Version). No significance was recorded in the effect of *Artemisia herba-alba* aqueous extracts on seedling total length and number of leaves in the case of *C. siliqua*. Significance appeared when roots length of *C. siliqua* seedlings were tested against concentration.

Table 2. ANOVA for the differences of means between stem total length, root length, number of seeds α = 0.05

Stem length (*cm*)		**Root length (*cm*)**		**No. of leaves**	
F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
2.57	0.127	21.1**	P < 0.01**	0.43	0.738

Based on results of Table (2), Tukey HSD test was conducted to compare between the effects of the treatments on root length of *C. siliqua* seedlings. As it explained in Table (3), comparing the effect of treatments on root length for *C. siliqua* seedlings revealed that all treatments have significant effect compared to control. However, no significant differences between both levels 25 % and 50 % nor between 50 % and 100%. Apparently, the later level of concentration had the highest significance in inhibiting roots to reach only 1.19 *cm* in average. Thus, it may be explanatory to study the regression analysis and understand the relation between tested concentrations and both root length and number of leaves of examined species.

Table 3. Tukey HSD test for the effects of treatments on the root length of *C. siliqua* seedlings

Concentration	**Root length (*C. siliqua* seedlings)**
	Means (*cm*)
Control	3.00^{a}
25%	2.00^{b}
50%	1.60^{bc}
100%	1.19^{c}
HSD	0.24

Figure (3) reveals fair regression between the only dependent variable in our experiment that is concentration and explanatory variable (Root length for *C. siliqua*). The coefficient of determination value (R^2) was 0.73 for root length and the model of regression can be expressed by bellow given equation:

$$R_L = 2.686 - 1.685\,C \qquad \text{.....................} (2)$$

Whereas: R_L is root length and C is concentration value

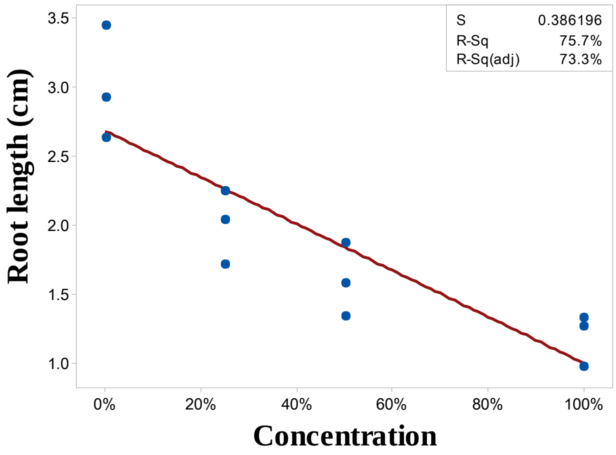

Figure 3. Regression between concentration root length for C. *siliqua* along with the associated coefficient of determination value.

4. Conclusion

The findings of this study, in general, go along with all conclusions cited in literature reviewed. It is noticed clearly that despite testing the *Artemisia herba-alba* aqueous extracts on different species (forestry, weeds and crops), similar results were obtained and reported. An extrapolation of these findings may be the key to the begging of adopting new management tools in the field of forest ecology.

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