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The Allelopathic Effects of Two Species of *Cistus* Genus on Germination and Root Length of *Ceratonia Siliqua* L

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Abstract: This study examines the allelopathic effects of *Cistus incanus (synonym C. villosus)* L. and *Cistus salviifolius* L. on the germination and the root length of *Ceratonia siliqua*. Six aqueous extracts were prepared for both species by soaking fresh leaves in cold water, boiling leaves and leaving for 24 hours, and grinding dried leaves. The result suggests that the germination percentage was slightly affected by extract concentration regardless of the type of extraction since F-value and P-value at 0.05 confidence level were 2.93 and 0.043 respectively. However, root length showed a significant response to the type of extraction, extract concentration, and interaction between both factors as F-values reached 6.3, 12.4, and 2.4 respectively. LSD test showed the response of seeds germination was inverse with the increase of concentration. In addition, it showed that the extracts of dried leaves were higher in root inhibition than the extracts of fresh leaves. The interaction effect reached its highest values when comparing the dried leaves' extract to the freshly soaked ones, even for the same concentrations. In conclusion, seeds of *c. siliqua* were able to easily start germination because the nutrition compounds needed were available in the endosperm, and the media supported them just by moisture for establishment. While for root length, the root tissue absorbed extra quantities of the inhibiting agents from media leading to failure or weakness in root development.

Keywords: Allelopathy; Aqueous extracts; *Cistus incanus*; *Cistus salviifolius*; Germination percentage; Root length.

INTRODUCTION

The term (Allelopathy) refers to the process of producing and releasing chemical substances by a plant into the environment (Rice, 1984; Hussain & Khan, 1988; Mallik, 2008). It seems that numerous sources of chemical substances exist in plants in different forms (Masoud and Omar, 2018). Some species depend on roots' ability to exert these chemicals. Or by dropping leaves or other areal parts to the adjacent soil (Thompson, 2005; Scognamiglio; 2013, Salhi et al., 2011; Idris and Omar, 2018). During the decomposition of the dropped matter, or by roots, and in the presence of water, some chemical compounds would reach to seed banks or the recently established seedlings. In many cases, the germination or seedlings' growth and development may fail or be inhibited (Idris and Omar, 2018).

The affected species would not respond to the allelopathic agent to the same extent. Herranz

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et al., (2006) reported that some Mediterranean species show reasonable levels of resistance towards the aqueous extracts of common allelopathic species. What is more interesting is the response of species to the chemical concentration. According to Omar et al (2017), the aqueous extracts of *Artemisia herba-alba* Asso have no significant effects on the germination of *C. siliqua* seeds, while the root length of established seedlings was significantly affected. Therefore, the natural regeneration in a given plant community may be affected by such allelochemicals. Such an influence may contribute to the shaping of forest ecosystems on the levels of composition, structure, and density (Caboun and Jacob, 2015).

In the Mediterranean region, many shrubs and annual plants produce some chemical substances in the act of competing against other species, especially in summertime (Muller, 1969). In Libya, natural vegetation covers about 1% of the country area, and 95% of it exists in Al-Jabal Akhdar in the northeast consisting of 100 - 140 species, subspecies, or varieties endemic to Al Jabal Al Akhdar (Radford et al., 2011). In such environments, allelopathy may, to some extent, influence biodiversity (Omar et al., 2017).

Cistus ladanifer L. has been reported as a fundamental allelopathic species to the extent that it may influence plant diversity in the Mediterranean (Chaves and Escudero, 1997; Chaves et al., 2001; Herranz et al., 2006). The current paper aims to investigate whether other species of the *Cistus*, which belong to the family of Cistaceae, could have any similar effect. Two native species were chosen: 1) *Cistus incanus* L. It is a shrub, erect or spreading with a stem up to 1 meter in length with white-villous. Leaves are ovate, obovate or elliptic, or almost flat. Pubescent or tomentose with stellate hairs. Flowers 4-6 cm in diameter, purplish-pink. Capsules densely adpressed-villous. The seeds are small and blackish (Jafri and El-Gadi, 1977). Figure 1 (A). 2) *Cistus salvifolius* L. It is an evergreen native small shrub, 60 - 90 cm in length. Leaves are green spreading and hairy, mostly ovate-elliptic, rounded at base, green, scabrid, and rugose above, with short stellate hairs on both surfaces, petiolate. The flowers are predominately white. Five sepals, manifestly cordate at the base, are scabrid with stellate hairs usually (Jafri and El-Gadi, 1985) Figure 1(B).

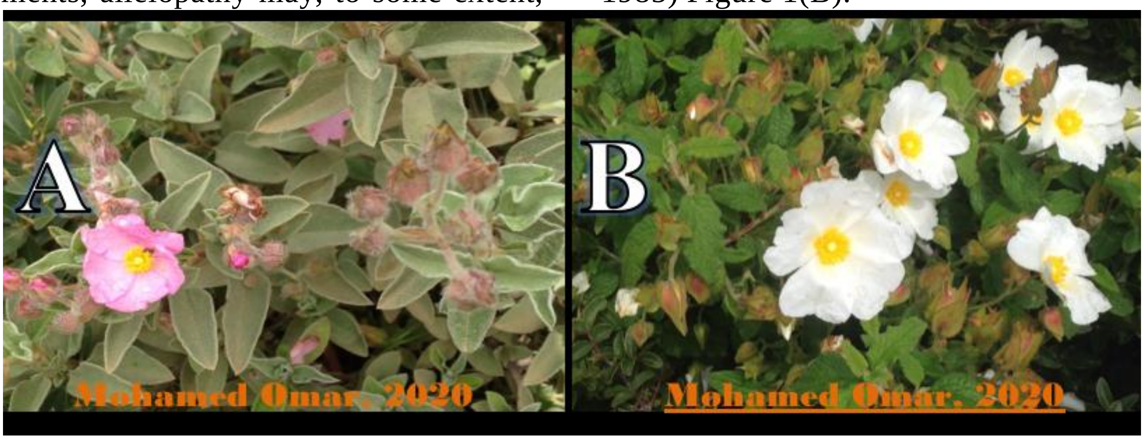

Figure (1). A) *Cistus incanus*, B) *Cistus salvifolius.*

The targeted species is *Ceratonia siliqua* L. which belongs to the Caesalpinioideae subfamily of the legume family, Fabaceae. It is an evergreen sclerophyllous tree or shrub, with a height of up to 9 meters. It can live up to 200 years (Masoud and Omar, 2018). Leaves are oval or elliptical, leathery, with a length of 10 – 20 cm. Flowers are greenish with red inflorescence, and inflorescence axis is catkin-like (Jafri and El-Gadi, 1985). *C. siliqua* seeds are known to have a high percentage of germination if they are subject to pre-sowing treatment; otherwise, they rarely exceed 10% (Bostan and Kilic, 2014). According to the same study, it seems that soaking the seeds for 30 minutes in sulfuric acid 95%, then immersing them in water for 48 hours, would increase the germination percentage to more than 90%.

MATERIALS AND METHODS

Preparing the aqueous extracts :Samples of *Cistus incanus* and *Cistus salvifolius* were collected in October 2019 from the forest of Werdama, located just about five kilometres east of Albayda city. Leaves were present in both species. Six aqueous extracts were prepared as follows: 1) Two extracts were prepared by collecting 100 gm of fresh leaves (on the day of samples collection) from both species, then soaked in cool distilled water for 24 hours at room temperature (Herranz et al., 2006). 2) Two extracts were prepared by collecting 100 gm of fresh leaves from both species (on the same day of collection), then added to boiling distilled water and left on a heat source for ten minutes, and kept for 24 hours at room temperature (Herranz et al., 2006). 3) Two extracts were prepared by drying fresh leaves for 30 days in room enrapture and then grinding dried leaves for both species with (Glen Creston Ltd, Stanmore, UK) mill. The powder of each species was diluted into distilled water 500 g to1000 ml (W/V %). The two mixtures were placed on a flask orbital laboratory shaker (mrc laboratory instruments, Cambridge, UK) for 24 hours at a speed of 120 rpm at room temperature (Ghorbani et al., 2008). Four-folded cotton fabric were used as a filter to separate rough solid particles from solutions. The solutions were then centrifuged with (ELE International, UK) at a speed of 2000 rpm for 15 minutes (Bajalan et al., 2013). All the above six mentioned extracts were considered main stocks. 25% and 50% solutions based on volume/volume percent (*v/v*. %) were prepared by adding distilled water from each main stock. All the prepared solutions and the main stocks were tested against control and among each other on *C. siliqua* seeds. The control is saline water to deviate from the osmotic-potential effect since the prepared solutions have a level of salinity. The salinity was l% (l g NaCl/1000ml distilled water), and the osmotic potential is equal to that in the high-concentration hot extract (Herranz et al., 2006).

Seeds preparation and testing : The seeds of *C. siliqua* were randomly selected and then subjected to a floating test. To break seed dormancy, a chemical treatment was applied. We then treated *C. siliqua* seeds with Sulphuric acid (95 %) for 20 minutes before soaking them in regular water for two days (Bostan and Kilic, 2014).

Experimental design and statistical analysis: A randomized complete block with a replication design was implemented. The six extract types were considered treatments, while the four solutions of different concentrations, from 0 % (control) to 100% main stocks, are considered blocks within each treatment. In every block, we prepared three replicants. Every replicant is a Petri dish (90 mm diameter) that contains five seeds of *c. siliqua* placed on a piece of filter paper, ensuring that seeds don't touch each other (Omar et al., 2017). The total number of dishes was 72. The Petri dishes were then incubated for ten days in a controlled cabinet at 20ºC in darkness (Memmert, Schwabach, Germany). Observations of germination percent for each dish and root length, as an average for every dish, were recorded. Concerning the statistical analysis, R (CRAN) software package was used. ANOVA test was applied, and the LSD test was carried out for the comparison between means. Some of our experimental units did not show any germination, meaning that the germination percentage was (zero). In such cases, data transformation is recommended. A logarithm-based transformation has been applied by replacing the value of each germination percentage (x) with $\log(x+1)$, as has been suggested by (Robert and Casella, 1999).

RESULTS

Seeds Germination: The germination started in the control block 48 hours from the beginning of the trial, reaching 95% in all treatments. Observations were recorded every 24 hours and the germination was tested in eight days (Abd El-Fattah et al., 2011; and Al-Watban & Salama 2012; Omar et al., 2017). The analysis of variance showed that there are no significant differences between the effects of aqueous extracts types, either the cold extract, the hot extract, nor the powder-based extract, on the germination of *C. siliqua* and for both species as the F-value was (1.61) as depicted in Table 1.

Table (1). Analysis of variance (ANOVA) for the extracts type and concentration on the Germination percentage.

S.O.V	D.F	S.S	M.S	F-value	P-value
Extract type	5	28.67	5.73	1.61^{ns}	0.177
Concentration	3	31.41	10.47	2.93*	0.043
Interaction	15	40.76	2.718	0.76^{ns}	0.711
Error	48	171.4	3.571		

Similarly, the interaction between the extract types and concentration has not shown any significance in its effect on the germination of seeds. However, the clear significance appeared among concentrations in a somewhat gradual inverse relation between concentration level and germination percentage, as shown in Table 2.

Table (2). Effects of *C. incanus* and *C. salviifolius* aqueous extracts on germination of *C. siliqua* seeds. α = 0.05

Concentration (%)	Means of germination percentage
Control	100^{a}
25%	60^{ab}
50%	45^{b}
100%	20^{c}
F-Value	2.93*
LSD	0.386

Means within columns followed by different letters are significantly different according to LSD, P<0.05.

Root length: Root length is a significant indicator for understanding phytotoxicity, that is, the extent to which chemical compounds affect the growth and development of plant cells. Moreover, it gave a better idea than the germination percentage to understanding allelopathies. The variance analysis showed a significant difference between the type of extract, the concentration, and the interaction on the root length (Table 3). The F value of concentration reached 12.4, which was the highest value, followed by the type of extract, which was 6.299. The F value of the interference was low; however, it was significant and reached 2.4.

Table (3): Analysis of variance (ANOVA) for the extracts type and concentration on the Root length

S.O.V	D.F	M.S	F-value	P-value
Extract type	5	30.72	6.299**	0.177
Concentration	3	60.33	12.369**	0.043
Interaction	15	11.86	2.439**	0.711
Error	48	4.87		

The LSD test showed (Table 4) that *C salvifolius* powder-based extract at a concentration of 100% has the highest significant effect on root length where the length of the roots exceeded 4 cm. Also, the interaction between type of extract and concentration is significant with a value ranging from 5 to 7.5 cm. This result was indicated whenever the type of extract differed and the concentration decreased. It can be concluded that the powder of the leaves gives a stronger effect than the soaked. The effect increases by rising the concentration except when treating the seeds with cold soaking for both types. At the same time, the length was from 7.5 cm to 8.5 cm, which is considered very close to the length of the root under the influence of control.

Table (4). Paired-wise comparisons between type of extracts, concentrations and interactions. Combinations that have same letters indicate to non-significant effect among them comparing to LSD. (A) Refers to shortest root lengths obtained ranged from (2.98 – 3.98) cm. (B) refers to longest root lengths obtained (3.99 – 5.22) cm.

		Cistus incanus aqueous hot extract				*Cistus incanus* aqueous cold extract				*Cistus salvifolius* aqueous hot extract				*Cistus salvifolius* aqueous cold extract				*Cistus incanus* powder-based extract			
		c	25	50	100	c	25	50	100	c	25	50	100	c	25	50	100	c	25	50	100
Cistus incanus aqueous cold extract	c	NS																			
	25	NS	NS																		
	50	NS	NS	NS																	
	100		NS	NS	NS																
Cistus salvifolius aqueous hot extract	c	NS				NS															
	25	A	NS			NS	NS														
	50	A	NS	NS		NS	NS	NS													
	100	A	A	A	A	A	A	A	NS												
Cistus salvifolius aqueous cold extract	c	NS				NS				NS											
	25	NS	NS			NS	NS			NS	NS										
	50	NS	NS	NS		NS	NS	NS		NS	NS	NS									
	100	A	A	A	NS	A	A	A	NS	A	A	A	NS								
Cistus incanus powder-based extract	c	NS				NS				NS				NS							
	25	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS						
	50	A	NS	B		A	NS	B		A	NS	B		NS	NS	NS	NS				
	100	A	A	A	NS	A	A	A	NS	A	A	A	NS	A	A	A	NS				
Cistus salvifolius powder-based extract	c	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS			
	25	NS	B			NS				NS	B			NS	NS			NS	NS		
	50	A	NS	NS		A	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		NS	NS	NS	
	100	A	A	A	NS	A	A	B	B	A	A	A	NS	A	A	A	3	A	NS	NS	NS
LSD	3.79																				

DISCUSSION

Regarding the germination initialization of *C. siliqua*, the key factor is the concentration of the active substance regardless of the species and the extraction method. The interpretation of such a difference in means has been reported by Chaves et al. (2001). Several chemical compounds are responsible for the inhibitory effect on the species that belong to the legume species. Among these substances are ferulic and p-hydroxybenzoic acid, which is the main substance that inhibits seed germination (Chung et al., 2001). It is unclear whether the other phenolic compounds have a direct inhibitory effect since (Herranz *et al.*, 2006) reported that some legume species such as *Lupínus hispanicus* did not show a significant response in terms of seed germination. However, Krogmeier and Bremner, 1989 reported that (caffeic acid, chlorogenic acid, and p-coumaric acid) have a clear effect when they reach soil on the seed germination of many species' seeds.

Root length is a significant indicator for understanding phytotoxicity, that is, the extent to which chemical compounds affect the growth and development of plant cells. Moreover, it gave a better idea than the germination percentage in understanding allelopathies. The result indicated that, whenever the type of extract differed and the concentration decreased. It can be concluded that the powder of the leaves gives a stronger effect than those soaked.

The effect increases by rising the concentration except when treating the seeds with cold soaking for both types. Masoud et al. (2018) concluded that the allelochemicals may be reducing cell division or auxin-induced growth of roots.

The aerial contains high inhibitory allelochemicals, and the extracts have inhibitory effects on the growth of roots and shoot some species. Also, the constituents of the aqueous extract have been reported to be potent inhibitors of seed germination and root seedling elongation (Zunino & Zygadlo, 2004; Masoud et al, 2018).

CONCLUSION

Root length is a significant indicator for understanding phytotoxicity which chemical compounds affect the growth and development of plant cells. These studies should be conducted further in more depth at the anatomical level, examining the toxic effect on tissues. This could represent a great opportunity to understand some variables in the plant environment, especially to understand the phenomenon of competition for limited resources in the ecosystem.

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التأثيرات الأليلوباثية لنوعين من جنس نبات البربش *Cistus Genus* على بذور الخروب *Ceratonia Siliqua L*.

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المستخلص : أجريت هذه الدراسة لبحث التأثيرات الأليلوباثية لـ *Cistus incanus (synonym C. villosus) L. Cistus salviifolius L*، على نسبة الإنبات وطول الجذر لـ *Ceratonia siliqua*. تم تحضير ست مستخلصات مائية للنوعين بنقع أوراق طرية في ماء مقطر بارد لمدة 24 ساعة، ومستخلص لأوراق طرية مغلية، ومستخلص عـن طريـق طحـن الأوراق المُجففة ثم إذابتها في ماء مقطر. تُشير النتائج إلى أن النسبة المئوية للإنبات تأثرت بشكل طفيف بتركيز المُستخلص بغـض النظر عن نوع الاستخلاص حيث أن قيمة F وقيمة P عند مستوى ثقة 0.05 كانت 2.93 و0.043 على التوالي. كما أظهـر طول الجذر استجابة معنوية لنوع الاستخلاص، وتركيز المستخلص، والتداخل بين كلا العاملين حيث بلغت قيم F 6.3 و12.4 و2.4 على التوالي. أظهر اختبار LSD أن استجابة إنبات البذور كانت في علاقة عكسية مع زيادة التركيز، بالإضافة إلى ذلك تبين أن مستخلصات الأوراق المجففة كانت أعلى في تثبيط الجذور من مستخلصات الأوراق الطازجة. وصل تأثير التداخل إلى أعلى قيمة عند مقارنة مستخلصات الأوراق المجففة مع المستخلصات المنقوعة حديثًا، حتى مع نفس التركيزات. نستنتج مـن الدراسة أن بذور *C. Siliqua* تمكنت من بدء الإنبات بسهولة؛ لأن مركبات التغذية اللازمة كانت متوفرة في السويداء، وكانت البيئة تدعمها بالرطوبة فقط للتأسيس. بينما بالنسبة لطول الجذر امتصت أنسجة الجذر كميات إضافية من العوامل المثبطة مـن الوسائط مما أدى إلى فشل أو ضعف في نمو الجذر.

الكلمات المفتاحية : Allelopathy، *Cistus incanus*، *Cistus salviifolius* ، طول الجذر، مستخلصات مائيـة، نسـبة الإنبات.

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