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Allelopathic Effects of Aqueous Extract from *Satureja thymbra* L. on Seed Germination and Seedling Growth of *Pinus halepensis* Mill. and *Ceratonia siliqua* L

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ABSTRACT

The objective of this study was to determine the impact of allelopathic potentials of aqueous extract of *Satureja thymbra* L. aerial parts on seed germination of *Ceratonia siliqua* L. and early growth seedling of *Pinus halepensis* Mill. and *Ceratonia siliqua L*. *Satureja thymbra* L. aerial parts were collected from Al-Jabal Al-Akhdar, Libya and collected aerial parts were dried and powdered. The treatments included 100%, 50% and 25% aqueous extracts along with water as a control. One-way analysis of variance (ANOVA) was performed and Regression analysis between treatments vs. root length, shoot length, and number of leaves among *Pinus halepensis* Mill and *Ceratonia siliqua* L. were done. The results showed seed germination of *Ceratonia siliqua* decreased by increasing extract concentration. The highest and lowest seed germination was belonged to control and 100 % extract respectively. In addition, the result showed a significant difference between treatments in root length and the number of leaves of both species. The LSD test showed that root length and number of leaves of *Pinus halepensis* Mill. and *Ceratonia siliqua* L. at treatments of 25%, 50% and 100% was significantly different from control. The regression analysis between root length, and number of leaves in *Pinus halepensis* Mill. and *Ceratonia siliqua* L. were significantly reduced with the increase in the extract concentration.
The results demonstrate that previously mentioned extracts have inhibitory effects on the growth of roots and shoots of *Pinus halepensis* Mill. and *Ceratonia silique* L. Thus, it is recommended to use from residual of medicinal plant or select them in crop rotation systems to weed by allelochemicals compounds.

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1. Introduction

Allelopathy is a phenomenon by which allelopathic plants release chemical compounds into the environment through root exudation, leaching by dew and rain, and volatilization or decaying plant tissues (Rice, 1984). Allelopathy is defined as direct and indirect effects of allelochemicals compound resulted from an organism, which may have inhibitor or stimulatory effects on the same or different organism (Putnam, 1985; Whittaker and Feeny, 1971). Allelopathy term was introduced as reciprocal effects of biochemical compounds among all plants and microorganism. More studies on allelopathy were conducted on germination percentage and speed and flowed by early seedling growth (Ben-hammouda *et al.*, 2001). Frequently allelopathy caused a decrease in plant growth more than what caused by competition in plants on sunlight, water, and nutrition (Rice, 1984). *Satureja spp* (family Lamiaceae) is found throughout the Mediterranean Basin, North Africa, the Canary Islands, South America, and West Asia. The genus *Satureja* consists of about 200 species, are native to sunny, dry, and rocky habitats. *Satureja spp* is annual or perennial plant and often aromatic (Cantino *et al.*, 1992). Wholly dried parts, typically above ground parts, contain medicinal values are used as remedies. The species rise up in temperate regions up to 45-60 cm high. They grow up in well-drained neutral alkaline dry soil. Over 30 species of this genus are distributed in eastern parts of Mediterranean area (Cantino *et al.*, 1992). In Libya, this genus is represented by only two species *Satureja thymbra* L. and *Satureja fortii* Pamp. The second being endemic to Libya. The two species could be found only in the Al-Jabal Al-Akhdar (Eastern Libya) (Jafri and El-Gadi, 1985). *Saturja thymbra* L. is a much branched, usually grey-puberulent dwarf shrub, and rise up to 20–35 cm high (Ball and Getliffe, 1972).

The essential oils isolated from various *Satureja spp* have shown various biological activities. Phenols, carvacrol, thymol, p-cymene, ß-caryophyllene, linalool, monoterpenes, sesquiterpenes, alcohols, phenolic acids, labiatic acids and flavonoids are the main components of the essential oils attained from the most *Saturja spp* (Sanchez, *et al.*, 1996).

The objectives of the present study were planned in order to test hypotheses that aqueous extracts of aerial parts of wild-grown *Satureja thymbra* L., which was collected from Al-Jabal Al-Akhdar-Libya, in varied concentrations have potential to inhibit the germination and seedling growth of *Ceratonia silique* L. and *Pinus halepensis* Mill.

2. Materials and Methods

2.1 Plant material

At the flowering stage (15 August 2017), the Aerial parts of *Satureja thymbra* L. were collected from Al-Jabal Al-Akhdar, Libya at localities near Al–Bayda city (590 m above sea level a latitude 32°74`612 N, and a longitude of 21°77`919 `E) (Fig. 1).

2.2 Preparation of aqueous extract

Aerial parts of *Satureja thymbra* L. were washed with water to remove any soil or other adhered material from their stems and leaves. Harvested aerial part (leaves, stems, and flowers) were dried at room temperature for one week. The air-dried plant material was ground into powder. The powdery material (100 g) was extracted with 500 mL distilled water. After 24 h, the extract was filtered through filter paper and placed on the Shaker for 24 hours. Then it was centrifuged at the speed of 2000 round per minute and for 15 minutes. The extract was passed through Whatman filter paper No.1. The obtained extract concentration was considered as the

foundation solution (100%). Then it was appropriately diluted with distilled water to give the final concentrations of 50% and 25% along with water as a control. The additional stock extract was transferred to a sterile glass container, and stored in the refrigerator at 4 C for future use.

Fig.1: *Satureja thymbra* L.

2.3 Germination experiment

Germination tests were conducted in Petri dishes (90 mm diameter) with four treatments by four replications (20 seeds per replication). Clean and sterilized Petri dishes were taken, and a piece of filter paper is placed over it. The filter paper in the Petri dishes was moistened by adding the different doses of the extract, and accordingly which acts as the medium for growth. The control plates were moistened with water but not overdamped. Five *Ceratonia siliqua* L. seeds of similar size were counted and positioned on the filter papers of each dish, ensuring that the seeds do not touch each other. Seeds of *Ceratonia siliqua* L. before used were immersed in 98% sulfuric acid for 20 min, then thoroughly washed in running tap water and dried on paper towels. The Petri dishes were then incubated for ten days in controlled cabinet at 20ºC in darkness. In the experiment, the germinating seeds were counted and the percentages of germination were measured.

2.4 Seedling experiment

Two weeks after planting, the *Pinus halepensis* Mill. and *Ceratonia siliqua* L. seedlings of similar size were counted with four treatments by three replications. Seedlings were irrigated with 3 ml of water (control) or 3 ml of different doses of the aqueous extract every day in all groups. At the end of the two weeks, the seedlings were carefully uprooted and washed thoroughly. The number of leaves, root length, a shoot length at harvest *Pinus halepensis* Mill., and *Ceratonia siliqua* L. seedling were recorded.

3. Statistical analysis

One-way analysis of variance (ANOVA) was performed to confirm the variability and validity of the data. Differences between the treatment means were compared using LSD test at 0.05% probability level. Regression analysis between treatments vs. root length, shoot length, and number of leaves among *Pinus halepensis* Mill. and *Ceratonia siliqua* L. were implemented. Change in the germination percentage with concentration of aqueous extract was evaluated using regression analysis for the combined data of *Ceratonia siliqua* L. All statistical analyses were done by SPSS.

4. Results

4.1 Germination Percentage

Effect of different aqueous extract concentrations of *Satureja thymbra* L. were significant on Seed germination of *Ceratonia siliqua* L. The results found seed germination of *Ceratonia siliqua* L. decreased by increasing of extract concentration. The highest and lowest seed germination was belonged to control and 100 % extract respectively (Table1).

Table 1. Effect of aqueous extract of aerial parts of *Satureja thymbra* L. on germination percentage of *Ceratonia siliqua* L. seeds.

Extract concentration (w/v)	Germination time(days) %				
	Day 2	Day 4	Day 6	Day 8	Day 10
Control	100	100	100	100	100
25%	35	65	100	100	100
50%	15	50	95	95	95
100%	0	5	25	35	35

The results of mean comparisons for effect of aqueous extract of *Satureja thymbra* L. on germination percentage of *Ceratonia siliqua* L. seeds clearly revealed that the two aqueous extracts of *Satureja thymbra* L. which are 25 and 50 %, stimulated the germination percentage of *Ceratonia siliqua* L. seeds. On the other hand, the 100%extract concentration of aerial parts of *Satureja thymbra* L. had great inhibitory effects on the germination percentage. The regression analysis between germination percentage and concentration of extract showed that 70.2% of variation in germination of the *Ceratonia siliqua* L. could be explained by the concentration of *Satureja thymbra* L. extracts (Fig. 2).

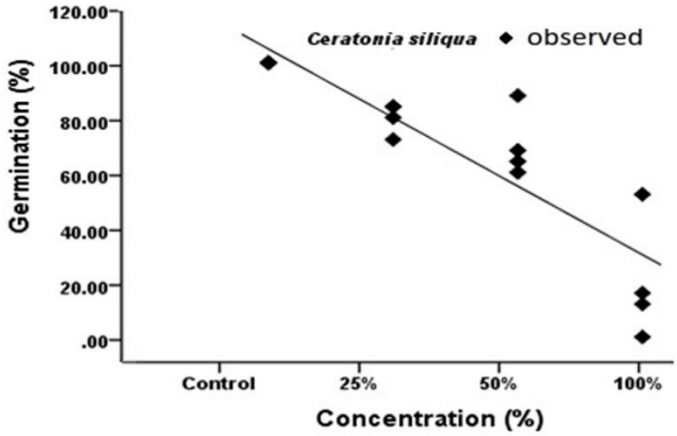

Fig.2. Regression analysis showing variation in seed germination of *Ceratonia siliqua* L. with different concentrations of aqueous extracts of *Satureja thymbra* L.

4.2 Seedling growth

ANOVA showed a significant difference ($p<0.05$) between treatments in root length and number of leaves of both *Ceratonia siliqua* L. and *Pinus halepensis* Mill. seedlings (Table 2). Between the two species, there was the highest reduction in the root length and number of leaves but there was no significantly different in shoot length. The LSD test showed that root length and number of leaves of *Pinus halepensis* Mill. and *Ceratonia siliqua* L. at treatments of 25%, 50% and 100% was significantly different from control (Table 3).

Table 2: Analysis of Variance (ANOVA) of root length, shoot length, and number of leaves in *Pinus halepensis* Mill. And *Ceratonia siliqua* L.under various treatments of aqueous extracts of *Satureja thymbra* L.

Plant species	*Pinus halepensis* Mill.		*Ceratonia siliqua* L.	
Parameters	F	P	F	P
Root length	5.708	0.02	9.910	0.005
Shoot length	1.981	0.195	0.483	0.703
No. of leaves	4.494	0.04	9.222	0.006

Table 3: Effect of aqueous extracts of *Satureja thymbra* L. on root length, shoot length, and number of leaves of *Pinus halepensis* Mill. and Ceratonia *siliqua* L.

	Pinus halepensis Mill.			*Ceratonia siliqua* L.		
Extract concentrations	Root	Shoot	No. of leaves	Root	Shoot	No. of leaves
Control	6.6a	6	14a	2.17a	9.9	5.4a
25%	5.86b	5.6	10.3b	1.38b	9.29	4.2b
50%	5.3bc	5.16	10.6b	1.22bc	8.51	2.6c
100%	4.66c	5.5	7.6c	1.09c	9.3	4db

Different letters in the table of the values in column indicate that the values are significantly different (p<0.05).

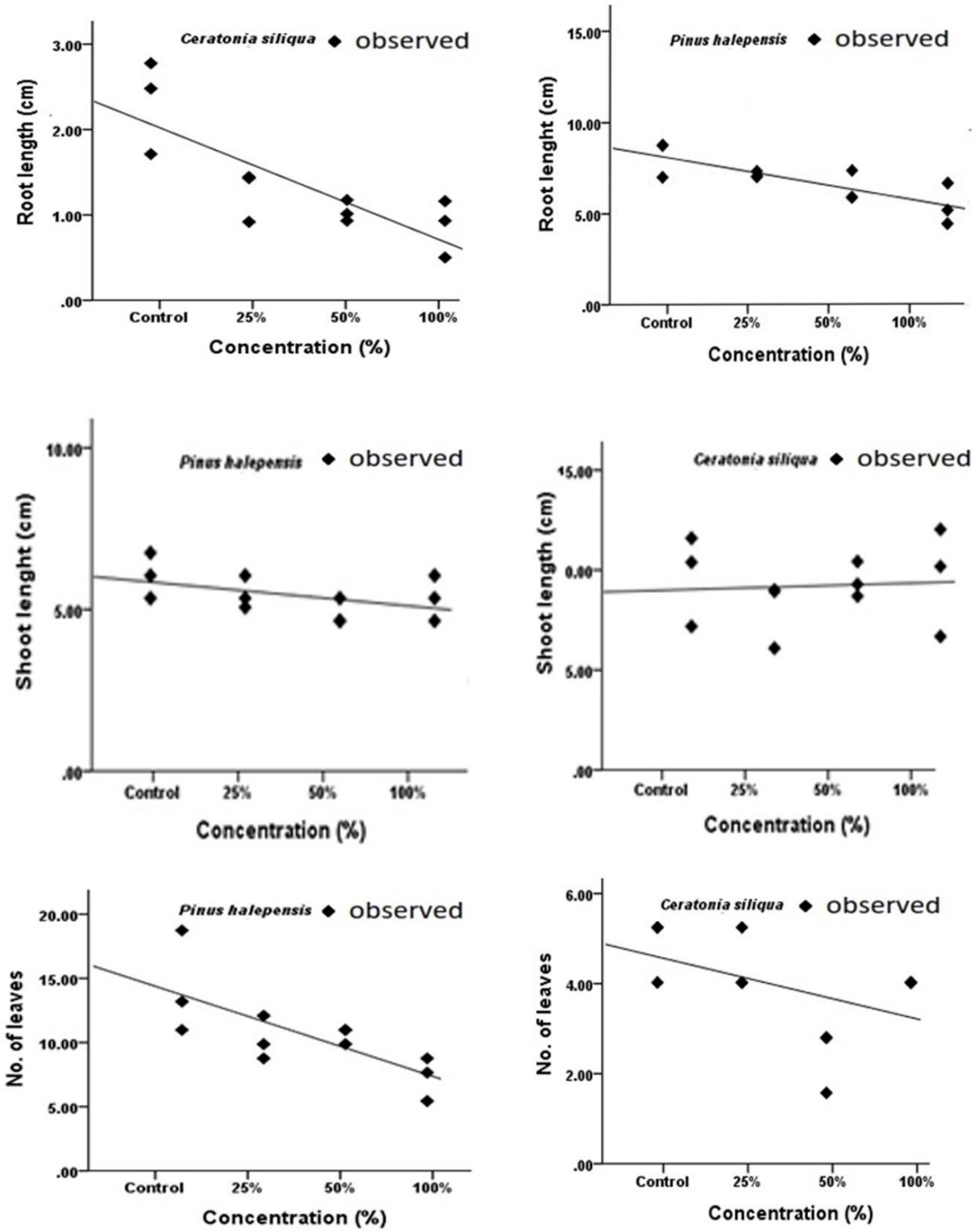

Fig.3: Relationship between different concentration of aqueous extracts of *Satureja thymbra* L. with root length, shoot length, and number of leaves of *Pinus halepensis* Mill. and *Ceratonia siliqua* L.

Regression analysis between treatments vs. root length, shoot length and a number of leaves among *Pinus halepensis* Mill. and *Ceratonia siliqua* L. showed the root length and number of leaves of both species declined with increasing concentration of the extract (Fig. 3), however at higher extract concentration, reduction was high for root length in the *Ceratonia siliqua* L. and for a number of leaves in *Pinus halepensis* Mill. (Fig. 3).

5. Discussion

The results of allelopathic effects of The *Satureja thymbra* L. aqueous extract on seeds germination of *Ceratonia siliqua* L., shown in Table 1, indicated that the inhibition of seeds germination increase with the increase of the aqueous concentration. This finding is congruent with the results of Ashrafi and Rahnavard (2008) who found that the degree of inhibition increased with increasing the extract concentration. Essential oils obtained from aromatic plants have been reported to exhibit herbicidal activity against seed germination (Muller *et al*., 1964; Vaughn and Spencer., 1993; Dudai *et al.*, 1999). The results have found the percentages of germination values were significantly reduced at aqueous concentration 25%, 50% and 100 % respectively compared with control, which was only water. Aqueous extract from different plants belonging to the Lamiaceae, Asteraceae, Myrtaceae and Verbenaceae families have been reported to have allelopathic properties (Verdeguer *et al*., 2009).

The regression analysis showed that there was a strong inverse relationship between the extract concentration and germination percentage. The aqueous extract concentrations of *Satureja thymbra* L. showed a significant inhibition of seed germination of *Ceratonia siliqua* L. in all the treatments compared to control (Table1, Fig. 2). Pirzad *et al*., (2010) found the different concentration extracts were significantly influenced final germination percentage, germination rate and index, root and shoot length, root/shoot ratio, fresh and dry weight of seedling of purslane.

From the LSD test, it was found that the shoot length in both test species at 25%, 50% and 100% extract concentration was not significantly different from control whereas at the same concentrations root length was significantly different from the control (Table 3). This indicates that root elongation was affected more than of the shoot. Inhibitory effects were on root elongation might be due to direct contact of root with the extracts. Not only the aqueous extract but also their constituents have also been reported to be potent inhibitors of seed germination and root seedling elongation (Zunino & Zygadlo, 2004).

The regression analysis between root length and number of leaves in both species were significantly reduced with the increase in the extract concentration (Fig. 3). Gholam *et al*. (2011) found that the irrigation of *Chenopodium album* L. and *Porulaca oleracea* L. seeds with extract solution of *Artemisia kopetdaghensis* Mill. and *Satureja hortensis* L. on at least for 15 days seems to have significantly inhibited at the growth of root and shoot, final germination, and rate of germination. Taban *et al*., (2013) reported that three *Satureja species* (*Satureja khuzestanica* Jamzad., *Satureja bachtiarica* Bunge. and *Satureja rechingeri* Jamzad.) exhibited potent inhibition activity on the seedling growth of Common cress (Lepidiumsativum L.), tomato (Licopersicon esculentum M.), and rye (Secale cereal L.).

The growth inhibition caused by allelochemicals released from *Satureja thymbra* L. may be due to its interference with the plant growth processes. The allelochemicals may be reducing cell division or auxin-induced growth of roots. Based on this study, it is apparent that the aerial contains a high inhibitory allelochemicals. The results demonstrate that previously mentioned extracts have inhibitory effects on the growth of roots and shoot of *Pinus halepensis* Mill. and *Ceratonia siliqua* L. Thus, it is recommended to use from residual of medicinal plant or select them in crop rotation systems to weed by allelochemicals compounds.

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